Mapping the self-incompatibility (S) locus in white clover (Trifolium repens L.)

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XVIIIth Meeting of the Eucarpia Fodder Crops and Amenity Grasses Section

10 – 14 May 2009

La Rochelle – France

Contents

Detailed program of the conference

Monday 11 May 2009.

8h30 - 9h30: Registration in the conference hall – Poster setting

9h30 – 10h00: Welcome talks (*Main Conference Auditorium)*

Claude Tabel, Chaiman of the ACVF (Association of French Forage Breeders)

Hélène Lucas, Head of the Inra Genetics and Plant Breeding Department

10h00-10h30: Introductive lecture: Grasslands and forage crops in France: context and stakes. Consequences for breeding. *C. Huyghe (France), C. Tabel (France)*

10h30: ***Session 1***: Genetic ressources:

Chairperson : C. Tabel, R2n, France

10h30-11h15: *Invited paper 1*: B. Boller (Switzerland), M. Vetelainen (Finland): A state of the art of germplasm collections for forage and turf species.

11h15 – 12h00: *Invited paper 2*: J.P. Sampoux, V. Badeau (France): Empirical niche modelling of the spontaneous diversity of forage and turf species to improve collection and *ex situ* conservation

12h00 – 12h40: Offered papers: **Session 1**

12h00-12h20: Evelin Willner, Susanne Hünmörder, Klaus J. Dehmer (Germany): Towards an enhanced utilization of plant genetic resources in grass breeding by characterization and evaluation trials

12h20-12h40: Metin Tuna, Asli Buyukbasar, Eyup Erdem Teykin, Hikmet Budak (Turkey), Toshika Yamada (Japan): Phylogenetics of genus *Dactylis* L. based on SSR marker analysis

12h40-14h15: Lunch

Plenary Session *Main Conference Auditorium*

Chairperson: O.A. Rognli, Norway

14h15-15h00: *Breaking method*: David Kopecký, Jan Bartoš, Adam J. Lukaszewski, James H. Baird, Vladimír Černoch, Roland Koelliker, Odd-Arne Rognli, Helene Blois, Vanessa Caig, Jaroslav Doležel, Andrzej Kilian: DArTFest - a platform for high-throughput genome profiling within the *Festuca – Lolium* complex

15h00: *Session 2*: Genetic changes in grassland and turf communities: which dynamics, questions to breeding.

15h00 – 15h45: *Invited paper*: I. Litrico (France), M. Vellend (Canada): Relationships between genetic and interspecific diversity.

15h45-16h30: Coffee break – Posters viewing

16h30-17h30: Offered papers:
Session 2 (Main auditorium)

16h30-16h50: Antje Rohde, Gerda Cnops, Joost Baert, Isabel Roldán-Ruiz (Belgium): Plant architecture in ryegrass – an alternative route to more persistent perennial ryegrass varieties

16h50-17h10: Athole Marshall, Michael Fothergill, Elaine Rees, Ellen-Sizer Coverdale (UK): Characterisation of variation in condensed tannin levels and persistence in *Lotus* spp.

17h10-17h30: Nadjette Missi, Jean-Louis Durand, Marc Ghesquiere (France): *Festuca*/*Lolium* specific markers for surveying genetic changes in Grasslands

Session 1 (Room Fernand Hervé, upstairs): Chairperson: J.M. Prosperi, Inra, France

16h30-16h50: Yuanhong Han, Christy M. Motes, Maria J. Monteros (USA): Evaluation and Utilization of Morphological Variation in a *Medicago truncatula* Core Collection

16h50-17h10: Bernadette Julier (France), Yasmina Semiani (Algeria), Meriem Laouar (Algeria): Genetic diversity in a collection of lucerne populations evaluated by SSR markers

17h10-17h30: Steve Hughes, Alan Humphries, Eric Hall (presented by Geoff Auricht): Collection and evaluation of Azerbaijan forage and crop genetic resources

16h00-17h30: Meeting of the Eucarpia board (Room Tabarly)

Bus transfer to town Center

18h00: Reception at the town hall

Guided visit of La Rochelle

Tuesday 12 May: Mid-tour visits

Departure at 8h15 in front of the entrance of the La Rochelle Aquarium

See details further in this brochure.

Wednesday 13 May

Plenary Session *Main Conference Auditorium*

Chairperson: R. Michaud, Agriculture Canada, Canada

8h30: ***Session 3***: Genetic progresses to meet end-users' expectations.

8h30-9h15: *Invited paper 1*: S. Van der Heijden (The Netherlands), N. Roulund (Denmark): Genetic gain in agronomic value of forage crops and turf: a review.

9h15-10h00: *Invited paper 2*: T. Gilliland (UK), V. Gensollen (France): Review of the protocols used for assessment of DUS and VCU in Europe. Perspectives.

10h00-10h45: Coffee break - Posters viewing

10h45-12h05: Parallel sessions of offered papers:
Session 3 (Main auditorium)

10h45-11h05: Barbara Chaves, Alex De Vliegher, Johan Van Waes, Lucien Carlier, Bram Marynissen (Belgium): Change in agronomic performance of *Lolium perenne* and *Lolium multiflorum* varieties in the past 40 years based on data from Belgian VCU trials

11h05-11h25: Aleksandra Bocian, Arkadiusz Kosmala, Marcin Rapacz, Zbigniew Zwierzykowski (Poland): The proteins involved in cold acclimation of perennial ryegrass (*Lolium perenne* L.)

11h25-11h45: Neculai Dragomir, Ioan Pet, Elena Pet, Carmen Dragomir, Sebastian Toth (Romania): Studies on several romanian agroecotypes of birdsfoot trefoil (*Lotus corniculatus* L.), with special reference to their productivity and forage quality

11h45-12h05: Louise Bach Jensen, Niels Roulund, Klaus K. Nielsen, Gerhard Deneken, Thomas Lübberstedt (Denmark): Application of molecular markers for variety protection in ryegrass (*Lolium perenne*)

Session 6: Other topics (Room Fernand Hervé, upstairs): Chairperson: D. Sokolovic, Serbia

10h45-11h05: Liv Østrem, Arild Larsen (Norway): Fiber content and plant development in *Festulolium*

11h05-11h25: Steven Van Hulle, Isabel Roldán-Ruiz, Erik Van Bockstaele and Hilde Muylle (Belgium): Comparison of different low-input lignocellulosic crops as feedstock for bio-ethanol production

11h25-11h45: Phillip Nichols, Andrew Craig, Amanda Bonython, Mary Jane Rogers, Tim Colmer, Ross Ballard, Nigel Charman, Ed Barrett-Lennard (Australia): Development of *Melilotus siculus* – a new salt and waterlogging-tolerant annual fodder legume species for Mediterranean-type climates

11h45-12h05: Nadia Elboutahiri, Imane Thami-Alami', El-Houssine Zaid, Sripada M. Udupa (Morocco): Physiological and genetic diversity in *Rhizobium sullae* from Morocco

12h05-13h30: Lunch

Plenary Session *Main Conference Auditorium*

Chairperson: M. Abberton, Ibers, UK

13h30: ***Session 4***: Molecular biology and biotechnologies for an appropriate management and creation of genetic diversity.

13h30-14h15: *Invited paper 1*: *I. Roldan-Ruiz (Belgium), R. Kolliker (Switzerland)* Marker assisted selection in forage crops and turf: a review.

14h15-15h00*: Invited paper 2*: *T. Yamada (Japan), L. Skot (UK)*: Allelic diversity for candidate genes and association studies: methods and results.

15h00-15h45: Coffee break – Poster viewing

15h45-16h45: Parallel sessions of offered papers :
Session 4 (Main auditorium)

15h45-16h05: Kerstin Diekmann, Trevor R. Hodkinson, Kenneth Wolfe, Rob van den Bekerom, Phillip J. Dix, Susanne Barth (Ireland): The complete chloroplast genome sequence of perennial ryegrass (*Lolium perenne* L.)

16h05-16h25: Stefano Capomaccio, Fabio Veronesi, Daniele Rosellini (Italy): Polyploidization and gene expression in *Medicago sativa*

16h25-16h45: Suresh Bhamidimarri, Malay C. Saha, Andrew A. Hopkins (USA): Understanding Summer Dormancy and Identifying Associated QTL in Tall Fescue

Session 3 (Room Fernand Hervé, upstairs): Chairperson: U. Fueurstein, Eurograss, Germany

15h45-16h05: Tim Vleugels, Joost Baert, Kurt Heungens, Marianne Malengier, Gerda Cnops, Erik van Bockstaele (Belgium): Resistance of red clover to broad spectrum of Sclerotinia trifoliorum

16h05-16h25: Franz Xaver Schubiger, Beat Boller (Switzerland) : The EUCARPIA multi-site rust evaluation – results 2007

16h25-16h45: Bohumír Cagaš, Magdalena Ševčíková and Radek Macháč (Czech Republic): Field resistance of *Festuca rubra* varieties to red thread (*Laetisaria fuciformis*)

16h45-17h45: Eucarpia section - General assembly

18h30: Visit of The Aquarium – Cocktail – Conference dinner at the Espace Encan

Thursday 14 may

Plenary Session *Main Conference Auditorium*

Chairperson: D. Kopecky, Czech Republic

8h30: ***Session 5***: Type and structure of varieties to better exploit genetic diversity

8h30-9h15: Invited paper 1: *Festulolium* hybrids: results, limits and prospects. Marc Ghesquière, Mike Humphreys, Zbigniew Zwierzykowski

9h15-10h00: Invited paper 2: Creation of heterotic groups and hybrid varieties. C. Scotti (Italy), C. Brummer (USA)

10h00-10h30: Coffee break – Poster withdrawal

10h30-11h30: Parallel sessions of offered papers:
Session 5 (Main auditorium)

10h30-10h50: Paolo Annicchiarico, Luciano Pecetti and Sandro Proietti (Italy) : Enhancing the adaptation to Italian environments of Egyptian lucerne germplasm for exploitation as a component of free-hybrids

10h50-11h10: Ulrich K. Posselt (Germany): Identification of heterotic patterns in perennial ryegrass

11h10-11h30: Dragan Milić, Slobodan Katić, Aleksandar Mikić, Đura Karagić (Serbia): Heterotic response from a diallel analysis between lucerne cultivars of different geographic origin

Session 4 (Room Fernand Hervé, upstairs): Chairperson: M. Monteros, Noble Foundation, USA

10h30-10h50: Lesley Turner, Sally O'Donovan, Sarah Hawkins, Christopher Macleod, Phil Haygarth, Chris Watts, Lawrence Clark, Richard Whalley, Andrew Binley, Mike Humphreys: Dissecting *Festulolium* chromosome 3 to locate rooting and drought resistance traits

10h50-11h10: Melanie Febrer, Michael T. Abberton, Glyn Jenkins, Dan Milbourne (Ireland): Exploring the potential for translational genomics

approaches in forage legumes: Regions of highly conserved microsynteny between white clover and *Medicago truncatula* revealed by BAC sequencing

11h10-11h30: James H. Baird, David Kopecký, Adam J. Lukaszewski, Jan Bartoš, Jaroslav Doležel, Andrzej Kilian, Robert L. Green (USA): Application of Diversity Arrays Technology (DArT) for genome profiling of turfgrass

11h30-12h15: General conclusions. Closing Ceremony.

12h15: Lunch

Program of the mid-tour (12th of May)

8h15:
Meeting place in front of the entrance of La Rochelle Aquarium
Participants board on four buses numbered from 1 to 4.
Morning visit will differ according to bus number (see details of visits further):

- buses 1 and 2 will visit a milk goat herd
- buses 3 and 4 will visit a beef cattle farm breeding a local bovine race.

Afternoon visits will be the same for the four buses, but for consistency of organisation, please stay in the same bus all day long.

8h30:
Departure of buses

10h00 – 11h15:
Morning visits:

- buses 1 and 2: milk goat herd farm (village of Vitré)
- buses 3 and 4: beef cattle farm (village of Saivres)

Both visit sites are mid-way between the cities of Niort and Lusignan

11h15:
Departure of buses to Lusignan

12h00 – 13h30:
Lunch time in Espace 5 at Lusignan

14h00 – 18h00:
Afternoon visits in research and breeding units around Lusignan (see details of visits further)

14h00 – 14h45:

- bus 1: Observatory of environmental research
- bus 2: National list registration trials of forage species
- bus 3: Breeding facilities of Jouffray-Drillaud company
- bus 4: INRA research unit for genetics and eco-physiology of forage species

15h00 – 15h45:

- bus 1: National list registration trials of forage species
- bus 2: Observatory of environmental research
- bus 3: INRA research unit for genetics and eco-physiology of forage species
- bus 4: Breeding facilities of Jouffray-Drillaud company

16h00 – 16h45:

- bus 1: Breeding facilities of Jouffray-Drillaud company
- bus 2: INRA research unit for genetics and eco-physiology of forage species
- bus 3: Observatory of environmental research
- bus 4: National list registration trials of forage species

17h00 – 17h45:

- bus 1: INRA research unit for genetics and eco-physiology of forage species
- bus 2: Breeding facilities of Jouffray-Drillaud company
- bus 3: National list registration trials of forage species
- bus 4: Observatory of environmental research

18h00:
Departure of buses to the site of 'Tumulus de Bougon'

18h30 – 19h30:
Visit of the Tumulus site (among the oldest Neolithic funeral monuments of Western Europe)

19h45 – 21h15:
Diner time in the cafeteria of the Tumulus site

21h30:
Departure of buses for the journey back to La Rochelle

Details about mid-tour morning visits

Milk goat herd - farm of Pascal MARET (Vitré)

In the Poitou-Charentes region (central-western France), the production and transformation of goat milk is of major economic importance. Livestock production systems contribute to shaping this region's attractive *bocage* landscape, a harmonious mix of hedges, pastures and annual crops. The goat milk cheeses are sold well beyond the regional and national borders and their quality enhances the image of this region. A varied group of stakeholders of the Poitou-Charentes milk chain have recently decided to work together in a research and development project called PaRMEELI that aims to analyse the environmental impacts of regional milk chains in order to identify improvement options across the entire supply chain from the farm and its inputs up to the transport of cheese to retailers. The farm which you will visit takes part in the project using life cycle assessment (LCA).

In fact Pascal MARET, who breeds 220 goats for milk and 40 cows for beef with 100 ha in the south of Deux-Sèvres, really wants to increase the energetic efficiency of his farm and to be as self-supporting as possible for feeding livestock. As an official of his cooperative, he knows that he has to find solutions to bring down the consumption of costly tasks through the supply chain. Pascal and his cooperative must achieve to withstand the ups and downs in the milk price in future. Nowadays, the main focus for the farmers and the industry is to improve competitiveness, coming close to sustainable development.

The farm of Pascal is a land-based mixed system, typical of temperate regions, combining rainfed crop production with livestock production based on the pasture of grasslands by cattle and the harvest of hay, straw and alfalfa. The zero grazing diet of goats implies good forage crops, with maize silage and fibrous dry matter from permanent and temporary grasslands. Due to agro-ecological conditions, which include low temperatures in the cold season and lack of moisture during summer, the system is open in terms of nutrient flow. Comparatively intensive, productions depend on use of external inputs: energy, fertilizers, and animal feeding stuffs.

La Belle à Celles-sur-Belle – F. Fuchs, octobre 2005.

Local breed beef cattle in organic farming - farm of Nicolas Durouchoux (Saivres)

The farm breeds a local bovine French breed (parthenaise breed), which is very appreciated for meet quality. The livestock includes 125 animals. Young beefs are produced under an organic farming production label, with an extensive use of grazing. The farm is settled on 88 ha, including 6 ha for cereal production and 82 ha of grasslands and meadows. Old permanent grasslands cover 20 ha. A large acreage of meadows has been sown with mixtures including tall fescue, ryegrass, timothy, red and white clover, alfalfa. The visit will include a view of the cattle and of sown meadows.

Details about mid-tour afternoon visits

Observatory of Environmental Research

The observatory for Environmental Research - Agro-ecosystem, Biogeochemical Cycles and Biodiversity (ORE-ACBB) is a unique design in Europe to investigate ecosystem function

Most of long-term experiments were not started with longevity in mind but rather to answer simple questions about nutrient requirements of crops. Later these long-term experiments have been used by scientists as research platforms to test the hypotheses, investigate mechanisms and develop or test models. Long-term experimental platform have been focused around the notion that certain processes such as changes in soil organic matter in response to land management and/or disturbance are long-term processes (years to decades). There are numerous examples in the literature in which data trends from short-term interpretations yield significantly different information from long-term analyses. The belief has been that the collection of data over long periods of time is necessary to permit generalizations and theory over sufficiently large spatial and temporal scales to evaluate ecological and biogeochemical consequences of land management and/or events such as drought, grazing, changes in trace gas fluxes...

Indeed, the observatory for environmental research - Agro-ecosystem, Biogeochemical Cycles and Biodiversity (ORE-ACBB) was established in France in 2005 and covering three different geographical regions: Poitou-Charentes (Lusignan), Auvergne (Theix-Laqueuille) and Picardie (Mons en Chaussée). This observatory is dedicated to monitoring long-term dynamics of ecosystems under anthropogenic pressures and its consequences on biogeochemical cycles. The basic scientific hypothesis of the ORE is that changes of system in response to anthropogenic disturbances are governed by long-term dynamics of soil organic matter. The ORE overall aim is to quantify internal and external environmental fluxes, to monitor changes of state variables of soil (C and N stock, microbial diversity and activity) and evaluation of resilience of soil-vegetation system according to contrasted management procedures applied for long-term period. Furthermore, coupling scales of time between process with short time steps and processes with longer time steps in a dynamic analysis is the basic criteria of this ORE, especially if we want to detect trends in ecological processes. Achieving the ORE-ACBB goals will help understanding the interplay of human and natural systems and forecasting the future states of ecological systems.

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Details about mid-tour afternoon visits

National list registration trials of forage species

In France, the National list registration trials are carried out by GEVES (Groupe d'Etude et de contrôle des Variétés et des Semences – Institute for evaluation and control of cultivars and seeds).

The GEVES is a public national institute supported by the French Ministry of Agriculture and the National Institute of Agronomical Research (INRA). With nearly three hundred research officers, technicians and administrative members of staff, GEVES evaluates each year about 1,200 new plant cultivars and tests about 80,000 seed samples in 12 sites over France.

GEVES is responsible for the studies required for:

- ✓ the registration of new plant cultivars,
- ✓ the legal protection of plant breeders' rights,
- ✓ the certification of seed lots for certain field crop species

The registration of a new cultivar on the National list is a required condition for commercialization. For registration, a new cultivar must be distinct, uniform and stable (DUS), and must provide some agronomic and technological advantages (VCU). GEVES performs technical evaluation on behalf of the CTPS (Comité Technique Permanent de la Sélection) under the authority of the French Ministry of Agriculture. New plant cultivars can be legally protected by the award of a plant breeders' certificate. GEVES acts on behalf of CPOV (Comité de Protection des Obtentions Végétales). There are many protocols and quality criteria for the production of certified seed. All seed lots of species subject to certification are sampled and evaluated. GEVES is responsible for the verification of variety identity and purity, and seed quality testing for most species, at the request of SOC (Service Officiel de Contrôle et de Certification).

On the site of Lusignan, the GEVES team includes three agents (1 research officer and 2 technicians). This team performs VCU (value for cultivation and use) trials in Lusignan for perennial and annual fodders, broad bean and lupins: about 1,400 plots and 500 observations lines of fodders, and an hundred plots of protein crops each year. DUS trials are also performed for lupins in Lusignan. Furthermore, the GEVES unit of Lusignan is responsible of the national VCU testing network of fodders, broad bean and lupins. These networks represent about 320 trials per year performed by 45 agents over France. Each year, about 60 and 80 new cultivars of fodder species and a few broad bean and lupin cultivars are assessed in these networks.

Details about mid-tour afternoon visits

INRA Research Unit for Genetics and Eco-physiology of forage species
(UR P³F)

The UR P³F research unit will propose participants to choose between two different visits. One visit will focus on field trial experiments about genetic changes under water deficit, agronomical value of species mixtures, and will offer a visit of a botanical garden, whereas the other option will present investigations on genetic changes in mini-swards, photo-morphogenesis and virtual plant modelling.

UR P³F first option:

Genetic evolution in a **Festulolium** *population under water deficit*

In a field trial established in Sept. 2007, we wish to assess to which extent genetic change occurs in a sward of perennial grasses. To do this, a tetraploid *Festulolium* hybrid population (cv. 'Lueur') is compared to its parent species (*L. multiflorum* and *F. glaucescens*), in pure stands or in 30:70 mixtures (resp.), under two water regimes, well-irrigated and rain-fed.

Change is expected to involve mostly interspecific genetic variability included in the *Festulolium* population, more specifically traits of drought tolerance inherited from *F. glaucescens*.

Water depth extraction is one of the most important traits accounting for persistency under drought. We use natural ^{18}O isotope abundance in plant and soil water, and joint variation of soil humidity (neutronic probe) to compare depth of water extraction between genotypes and water treatments over seasons and years. Genetic changes are estimated through variation of the frequency of species-specific markers, - at the population level by leaf random sampling in plots, and - at the individual level, after extracting plants from the plots, marker genotyping and cloning for further assessment in nursery under isolated conditions. Adjacent plots sown in Sept. 2008 will investigate how sexual reproduction and traits related to fitness may also accelerate gene evolution in swards under water deficit following natural seed scattering and the replacement of the most drought-susceptible (e.g. *Lolium*-like) individuals.

Agronomical value of meadow seed mixtures including a high inter-specific diversity

A field trial was sown in 2003 to test: (i) if there is a relationship between the number of species entering a meadow seed mixture and the agronomic value of the meadow, and (ii) to assess how changes with time in species composition affect forage yield.

Twenty five different species compositions, including from one to eight species, are compared. Some compositions include only grass species, whereas others include grasses and legumes. The grass species entering the compositions are *Lolium* species, *Dactylis glomerata*, *Festuca arundinacea*, *Festuca rubra*, *Poa pratensis*, *Phleum pratense* and the legume species are *Trifolium pratense*, *Trifolium repens*, *Lotus corniculatus*, and *Medicago sativa*.

The twenty five species compositions are evaluated for two cutting rates (cutting each 30 days and each 50 days) and two nitrogen supply levels (60 kg/ha and 160 kg/ha for grass only mixtures, no supply and 30 kg/ha for mixtures including grasses and legumes).

The botanical garden

The Botanical garden gives a view of the grass and legume species growing spontaneously in France in natural meadows and grasslands, and of the main species sown in France as forage and turf grasses. It includes 70 grass species and 44 legume species.

Spontaneous species are arranged in several beds corresponding to different natural swards, and other beds gather collections of sown forage and turf species.

UR P³F second option:

Genetic and eco-physiological determinants in changes of a perennial ryegrass population under frequent and infrequent defoliation

Forage meadows consist of many plants of different genotypes and often different species. Moreover, meadows are submitted to environmental and management constraints such as defoliation. The resulting effect of the interaction between these different constraints is a decrease of the agronomical value of meadows over time.

The aim of this study is to understand the genetic and eco-physiological determinants involved in the change of a mini sward of perennial ryegrass under frequent and infrequent defoliation. This includes three main objectives: i/ to evaluate the role of phenotypic plasticity and selection in the changes, ii/ to identify genomic regions involved in the genetic changes and iii/ to identify environmental factors affecting the changes. In order to achieve these objectives, a perennial population including a great variability for its aboveground morphology was surveyed under two defoliation rhythms over three not-overlapping generations. Survival and aerial morphogenesis were characterised for each individual plant. Fitness was taken into account to define the number of siblings per genotype for the following generation. Allelic frequencies of loci across the genome were followed by using AFLP and SSR markers. The allelic frequency evolution was assessed for QTLs of traits involved in aboveground morphogenesis. Due to differences in plant morphology there is variability in light intercepted by individuals. Therefore, we attempted to evaluate this light interception variability by developing an approach based on simplified 3D representation of individuals. Then we analyzed dynamics of light interception by taking into account incident light for the two defoliation regimes and density treatments. Difference in plant morphology induces some plants to be shaded by others and consequently to undergo regimes of light quality differing from plant in full sun. One of the light quality component is the blue radiation. It is reduced in shaded areas because of the optical properties leaves. One of the effects of the blue-poor light is an increase of the leaf elongation rate. We analysed the photo-morphogenesis mechanism by investigating how interception of blue radiation signal occurs to give rise to a response. In an attempt to improve our understanding how these mechanisms are integrated in functioning of plant canopy, we developed a virtual plant modelling based on architectural 3D representation of individuals.

Details about mid-tour afternoon visits

Breeding facilities of Jouffray-Drillaud seed company

During the visit at Jouffray-Drillaud Research Facilities, you will have a brief presentation of the company and a presentation of the species bred in La Litière.

Jouffray-Drillaud is the result of joint venture of two old family companies in 1967. The company is owned by the cooperative Terrena since 2001. Jouffray-Drillaud is now one of the most important forage and turf Seed Companies in France with 140 employees and 4 facilities. The turnover is around 62 million euros.

The Research station in La Litière, Saint Sauvant, is established on 150 acres. 4 breeders and 10 technicians are involved on forage grass and legumes breeding (ryegrass, tall fescue, cocksfoot and alfalfa,…), turf grass breeding (ryegrass, tall and fine-leaved fescues, …), cover crops breeding (rye, phacelia, …), pulse (white lupins, vetches) and Biofuel breeding (Reed canary grass, switchgrass).

Following the presentation of the company and the Research station, we will focus the visit on turf breeding plots, with a special attention on the genetic diversity used with the aim of developing low input turf, birdsfoot trefoil trials, reed canary grass and switchgrass breeding nurseries for biofuel production.

Site of 'Tumulus de Bougon'

The site of 'Tumulus de Bougon' is the oldest necropolis known in Europe (built 4700 years before J.C). It includes six wide tumuli, built on the basis of elaborated dolmens, whose shape suggests mysterious proto-pyramids. A visit of the tumuli will be proposed with comments by English-speaking guides.



Book of abstracts

Section 1: Genetic resources

Section 2: Genetic changes and dynamics in grassland and turf communities.

Section 3: Genetic progresses to meet end-users' expectations

Section 4: Molecular biology and biotechnologies for an appropriate management and creation of genetic diversity

Section 5: Type and structure of varieties to better exploit genetic diversity

Section 6: Other topics

Grasslands and forage crops in France: context and stakes. Consequences for breeding

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In a first part, the paper will present the socio-economic context of grasslands and forage crops in France over the last decades. The grasslands and forage crops contribute 45% of the total French arable land. Variation in acreage of the various types of soil covers in the various regions will be presented with a special attention to temporary grasslands. The variation in the size of herbivore herds will be described, and especially the number of dairy cows and suckling cows. Data related to seed production of forage species will be presented.
The second part will present the main stakes for the future of grasslands. They are related to i) balance between income and workload for the farmers, taking into account the common agricultural policy, ii) quality of animal products in response to end-users' expectations and iii) combination of economic performance and environment preservation, with a special interest to reduction of soil and nutrien losses, reduction of fossil energy consumption and greenhouse gas emission and hosted biodiversity.
Meeting these stakes assigns new goals to breeding. It first requires an increased persistence of grasslands stands which may be achieved through either more persistent plants or through exploitation of the population dynamics of swards. It also offers good prospects for mixtures of species as sources of overyielding and more yield persistency. Finally, these stakes offer new prospects for forage legumes, source of nitrogen fertility in cropping systems and protein in animal diets with a positive effect on the grassland biodiversity.

Invited paper 1

A state of the art of germplasm collections for forage and turf species

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Abstract
Four categories of plant genetic resources (PGR) are important for breeding: Wild relatives, ecotypes, landraces, and cultivars. Fodder crops and amenity grasses differ from field crops in the relative importance of these categories, as well as in the relative importance of in situ vs. ex situ conservation. As they are less domesticated, a continuum of wild and naturalized forms of the cultivated species of fodder crops and amenity grasses exist as ecotypes in a great variety of permanent grasslands. An often random fraction of this variety has been either used to aliment active breeding pools or collected in gene banks, while a great range of potentially useful genetic variation remains yet to be exploited.
This paper reviews recent, partly molecular marker based literature pointing to criteria and strategies of collecting PGR in situ in grassland dominated regions. Guidelines are given for establishing and maintaining ex situ germplasm collections and for evaluating them in view of their utilization by breeders. A comprehensive overview of publicly accessible germplasm collections, especially those in the auspices of European Co-operative Programme for Plant Genetic Resources (ECPGR) is given. Their present status and expected future developments are high-lighted. Strategies for breeders to utilize domestic and exotic germplasm in breeding programmes are presented. Also pre-breeding strategies along with a Nordic case study will be presented in order to demonstrate the utilisation of unique alleles outside the breeding pool. The potential of new molecular tools for the management and better utilization of PGR collections is shown.

Invited paper 2

Empirical niche modelling of the spontaneous diversity of forage and turf species to improve collection and *ex situ* conservation.

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Abstract

Rational sampling of the spontaneous diversity of forage and turf species requires an *a priori* knowledge of the range of environmental conditions suitable for these species. We introduce some concepts and methods for investigating the environmental range of species by empirical modelling of species ecological niche, and we suggest how such investigations could help to plan collection campaigns and to improve the choice of core-collections. The empirical modelling of the ecological niche of a species consists on building a function of environmental parameters predicting the presence of the species from a calibration dataset including observed presence-absence or abundance records of the species and environmental data at observation sites (Guisan and Thuiller, 2005; Araujo and Guisan, 2006). We emphasize that data from collection campaigns of plant breeders are valuable information for niche modelling. We introduce two methods for investigating the environmental distribution of species and for niche modelling based on presence-absence data: the canonical correlation analysis (Gimaret-Carpentier et al., 2003) and the logistic regression. We give examples combining niche model and GIS software that may contribute to organize collection campaigns. We suggest that models predicting probability of presence of species may be useful for the selection of core-collections. Such models may help to delineate geographically isolated areas of presence of species that should be sampled separately for selecting a core-collection. In each isolated area of presence, we propose to stratify the accessions in clusters according to the predicted probability of presence of the species in collection sites, and to select accessions in each cluster.

Keywords: core-collection, GIS software, niche modelling, spontaneous diversity

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P1

The genetic diversity of fine–leaved fescue (*Festuca* L.) species in Lithuania

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Abstract

Fine-leaved fescue (*Festuca* L.) species are valuable breeding material with potential use for marginal land cover. However the breeding of these species is complicated by morphological similarity among species and high heterogenity within species. Molecular DNA markers are needed to facilitate species identification. Inter simple sequence repeat polymerase chain reaction (ISSR-PCR) was used for the genetic analysis of the five fine–leaved fescue species encountered in Lithuania, namely *F. sabulosa* (Anderson) H. Lindb., *F. polesica* Zapał*, F. ovina* L., *F. trachyphylla* (Hack.) Krajina and *F. psammophila* (Hack. ex Čelak.) Fritsch. Fifty six ISSR markers were scored for 21 fescue accessions. Similarity indicies (Lynch, 1990) were calculated and UPGMA dendrogram constructed with NTSYSpc 2.2. Three species (*F. ovina*, *F. trachyphylla* and *F. psammophila*) could be distinguished as separate clusters in the dendrogram. Further analysis revealed two ISSR fragments of 600 and 950 bp to be species-specific for *F. psammophila*. These fragments are potential targets for specific SCAR marker development. The remaining two highly related species (*F. sabulosa* and *F. polesica*) formed one intermixed cluster and could not be distinguished from each other. Ploidy analysis was also performed to validate the ploidy level of the investigated species which confirmed the hexaploid chromosome number for *F. trachyphylla* while all other species were diploid. The results of this work show the suitability of the ISSR method to investigate genetic diversity of fine-leaved fescue species encountered in Lithuania and provide valuable data for the identification of these species.

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P2

Genetic Variation in Lowland Switchgrass ((*Panicum virgatum* L.)

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Abstract

Switchgrass (*Panicum virgatum* L.) is a second-generation feedstock identified for use in cellulosic ethanol production that performs better than corn and soybean in terms of its environmental impact (Scharlemann and Laurance, 2008). Understanding genetic variation for biomass yield in switchgrass would be helpful in determining the appropriate breeding approach for cultivar development. The objective of this study was to estimate the genetic component of variation in lowland switchgrass. A total of 30 full-sib families were produced in greenhouse in the spring of 2007 using nested design (NC design I) by crossing 10 genotypes, each with 2 to 4 other genotypes, both selected at random. The parental genotypes were selected from elite breeding populations and PI collections. The seedlings of these 30 families along with other seventeen full-sib families, and two checks; Alamo and Blade™ EG1101 were raised in a greenhouse in the summer of 2007. These families were transplanted in autumn of 2007 at two Oklahoma locations, Ardmore and Burneyville, using the R49 honeycomb design with 1.5m plant-spacing. Each family was represented by 30 genotypes at each location. The biomass from individual plants was harvested separately after the killing frost in 2008. Genetic components of variation were estimated following mixed model in SAS and heritability was estimated according to Gallais (2003). Results from Ardmore location showed a significant general combining ability, while specific combining abilities indicated that both additive and dominant gene actions were important in biomass dry matter yields of lowland switchgrass. The heritability estimate (~0.2) based on family analysis as well as parent-progeny regression was low suggesting that the trait was under the control of many genes with minor effects.

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P3

Morphological and molecular diversity of branching in red clover (*Trifolium pratense*)

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Mixed grass-clover grasslands are an essential element of sustainable farming systems. The presence of clover in the mixture contributes significantly to the reduction of nitrogen fertilizer application needs, and results in improved nutritional value. Plant architecture is under genetic and environmental control and we can anticipate that it will strongly influence traits such as forage yield, re-growth capacity, seed yield and persistence in fodder crops. The genetic aspect of branching has been widely studied in model plants but did not obtain much attention in the past in red clover. Our aim is to translate knowledge on genes involved in meristem initiation, bud formation, and the activity and determination of the apical meristems from model plants to red clover.
For this study we are building a collection of genotypes derived from commercial cultivars, landraces and natural populations and covering a broad-range of variation for branching present in red clover. Phenotyping carried out in 2008 has shown a huge diversity for branching both, between and within populations. In a further step we will investigate the relationship between branching patterns and other important traits in red clover. Analyses of allelic diversity in branching genes will allow us to determine the genetic factors which are responsible for architectural differences in this species. Our final goal is to translate this genetic knowledge into breeding applications to produce red clover cultivars with higher dry matter and seed yield and improved persistence.

P4

Characterization and nutrition value evaluation of Polish perennial ryegrass ecotypes

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Abstract
Perennial ryegrass (*Lolium perenne* L.) is one of the most important cool-season species in many countries. It is valued for high yield potential, fast establishment, reduced tillage renovation applications. It may be used in dairy and sheep forage systems. The quality of perennial ryegrass cultivars depends mainly on resistance to snow mould caused by *Fusarium* spp. and rusts resistance caused by *Puccinia* spp. Efforts to reduce losses caused by *Fusarium* spp. and *Puccinia* spp. are mainly based on the use of resistant varieties. New germplasm with the resistance and other characteristics on the level (or higher) of commonly used cultivars, are needed for breeding of new cultivars and for improving those which are already used. Because of this agronomic value of 12 Polish ecotypes were investigated during three years. Their nutrition value was analyzed according to methods described by Marco Meisser et al. (Agroscope Changins-Wadenswil Research Station ACW). Green and dry mass production, organic matter, digestibility of the organic matter, digestibility of crude protein, NEL, NEV, EM, sugar content and the main constituents of the cell wall (crude fiber, ADF, NDF and lignin) was determined. During the second year of the experiment green and dry mass production and nutrition value of 4 ecotypes was higher or on the level of Polish control variety Argona (green and dry mass production: 12,10 dt/ha and 4,10 dt/ha, respectively). During the third year of the experiment green and dry mass production and nutrition value of 6 ecotypes was higher or on the level of Argona (green and dry mass production: 16,20 dt/ha and 5,5 dt/ha, respectively). Those ecotypes will be included into breeding programmes.

P5

Microsatellite Diversity in Swedish Timothy

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Abstract
The use of timothy as a forage crop in Sweden can be traced back, at least, to the beginning of the 19th century. Now it is the most important forage crop in Sweden due to its adaptation to the cold and humid climate. The short breeding history and the rapid reduction of historical grasslands, may incur losses of genetic diversity of importance for future breeding.
At the Nordic Genetic Resource Center (NordGen) a large collection of timothy is preserved *ex situ*. A majority of these accessions are composed of samples collected from natural populations.
As a part of a Nordic project funded by the Nordic Joint Committee for Agricultural Research this study seek to improve the knowledge about one of the most important forage crops in the region, through both phenotypic and genotypic characterization of the Nordic timothy collection. Field trials are established and molecular analysis (functional genomics and diversity studies) are ongoing in four of the Nordic countries. Our primary aim is to describe the diversity within and between populations in a subset of the collection currently available at NordGen, and thereby facilitate use of the material for breeding.
We have evaluated about a third of the 355 markers developed in 2003 by Cai et al., out of these we have chosen 20 for further analysis. This study indicated a lower diversity within the commercial variety than the wild populations.

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P6

Genotypic differences in symbiotic N_2 fixation of some alfalfa (*Medicago sativa* L.) genotypes

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Abstract

Alfalfa (*Medicago sativa* L.) is important forage crop with the ability of nitrogen fixation which offers possibilities for participation in sustainable agriculture. Different alfalfa genotypes differ in efficiency of symbiotic nitrogen fixation. Furthermore, various rhizobial strains differ in nitrogen fixation capacity and it is therefore possible to select more efficient strains. The nitrogen fixation by legume-rhizobium association is genetically complex. Phenotypic differences can be brought about by genetic variation in either the plant or the bacterium and both symbionts. In order to determine favourable gene combinations, coincidental and coordinated selection and breeding of both symbionts is required.

In highly controlled laboratory experiment, four alfalfa genotypes were grown in all possible combinations with five strains of *Sinorhizobium* spp. with the aim to access differences in nitrogen fixation among alfalfa genotypes and compatibility of symbionts. Based on shoot dry weight, total and fixed N, it was noted that each genotype showed a great compatibility with particular strains. Variety K-28 inoculated with all investigated strains exhibited the highest shoot dry matter compared to other investigated genotypes. Using symbiotic N fixation, variety K-28 inoculated with L3Si strain gained high compatible association due to the 86% of shoot dry weight in relation to uninoculated control with full N content (100%). The significant coefficient of variation (CV%) for shoot dry matter of inoculated treatments and uninoculated controls was found 19.4-57.8 and 14.7-24.9, respectively depending on bacterial strain and plant genotype. Similar results were found for total N content. Higher values CV% of inoculated plants compared to uninoculated plants indicated the influence of strain on increasing variability of the plants. As the effectiveness of the N fixation varies widely in different rhizobia-host combinations, it would be possible to identify the highly effective rhizobial strains, which would represent commercial strains of microbiological fertilizers for particular cultivars.

P7

Collection and research on wild forage populations as a source of useful traits

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The wild flora is a rich repository of wild forage species, which are valuable source of genes for varieties improvement and adaptation to changing environmental conditions. In wild forages variability appears especially in its general morphological characters, but high variability also exists for specific agronomic traits, including herbage regrowth and quality, flowering habit or insect resistance. Since 2002 the research targeted at evaluation of morphologic, agronomic and forage quality traits of some forage species collected from diverse habitats of Slovakia and foreign countries has been conducted. Wild populations of *Medicago* sp., red clover and bird's foot trefoil were evaluated in a field experiments at the experimental station of Research Institute of Plant Production in Piešťany. Considerable variation between and within wild populations was observed for most of evaluated traits. The wild populations of bird's foot trefoil and red clover were morphologically differentiated from the cultivated varieties; they were characterized by plants with prostrate and semi-prostrate growth habit with creeping thin stems. The prostrate growth habit of some wild populations was connected with higher percentage of plants survival. The wild populations showed outstanding health condition when compared with commercial varieties. The agronomic performance of wild populations was lower when compared with evaluated varieties. Nevertheless, a remarkable variability occurs within wild populations and the best ones may challenge the control varieties. Crude protein contents and mineral concentration of natural *Medicago* sp. populations were higher in comparison with control varieties. The investigation of association between morphologic traits of bird's foot trefoil and geographic characteristics showed that the phenotype of wild populations was connected with latitude, longitude and elevation of collecting sites. The results indicate possibility of utilization wild populations in research and breeding of forages for specific environments.

P8

Evaluation of some Moroccan collection of cocksfoot for summer dormancy

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Abstract

Summer drought is one of the most factors affecting the persistence of perennial grasses in rainfed Mediterranean areas, resulting in underutilisation of the perennial grasses in the forage cropping system. Summer dormancy is one trait that could improve drought survival and autumn recovery of the perennial forage species. For this purpose, evaluation of summer dormancy and other agronomic characteristics of sixteen orchardgrass ecotypes, collected from different regions of Morocco was undertaken in the experiment field. As a summer dormancy control, we used the cultivar Kasbah, while the summer active cultivar was represented by Medly. Results showed a good level of summer dormancy of most Moroccan ecotypes. Summer dormancy was correlated with drought survival after three summer drought seasons. Furthermore, the most summer dormant ecotypes and Kasbah had also high level of persistence. The promising material will be included in further breeding programmes.

Key words: orchardgrass, summer dormancy, drought survival, Moroccan ecotypes

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P9

Agronomic evaluation of Moroccan ecotypes of tall fescue

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Abstract

Drought or reduced water availability is the main factor limiting crop production in Mediterranean area. The aim of this study was to select ecotypes of *Festuca arundinacea* Schreb. adapted to the Moroccan environment. During three years, eleven ecotypes of tall fescue, collected from different regions of Morocco and seven commercial varieties were evaluated for the following agronomical traits : dry matter yield, summer growth, heading date, survival rate, plant height and number of tillers.

A factorial correspondence analysis (ACP) showed that three components explain 80% of total variability. Dry matter yield, plant height, number of tillers and summer growth were associated with the first component. Heading date was strongly associated with the second component, while survival rate was associated with the third component. Three groups of tall fescue and one independent lineage were defined from the cluster.

Dry matter yield was well correlated with plant height, number of tillers and summer growth ($r = 0.68$, $r = 0.61$ and $r = 0.56$ respectively).

In term of production potential and perenniality, some local ecotypes present certain superiority in comparison to the commercial varieties. These ecotypes could be eventually exploited to create new Moroccan varieties.

Key words: tall fescue, evaluation, perenniality, ecotypes.

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P10

Genetic structure of local populations and cultivars of meadow fescue from the Nordic and Baltic regions

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Abstract
The genetic diversity within and among 81 local populations from Norway and Lithuania and 26 cultivars of meadow fescue (*Festuca pratensis* Huds.) from Norway, Sweden, Finland, Denmark, Lithuania, Latvia and Estonia was investigated using AFLP markers. Genetic diversity was high with nearly 70% of the molecular variation within populations. Principal coordinates (PCO) analyses indicate that local populations from Norway and Lithuania consist of both older natural populations and naturalized populations established recently by migration from sown meadows. This is based on the fact that naturalized populations cluster with cultivars from the respective countries. The PCO-analysis separates the natural local populations from Norway and Lithuania. They belong to different gene pools and reflect dispersal from different glacial refugia after the last glaciation. The Nordic and Baltic cultivars are very similar (6.25% of variation between groups), and the Baltic cultivars are very homogenous with 91% of variation within the cultivars, indicating narrow breeding populations of this species in the Nordic-Baltic region. All cultivars are most closely related to local populations from the Baltic. The results can be used to improve management and utilization of gene bank resources of meadow fescue. Conservation of gene bank accessions should focus on those local populations least influenced by gene flow from cultivated meadow fescue since they probably represent pure natural populations.

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P11

Genetic diversity in tall fescue using AFLP markers

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Abstract

Despite some data on tall fescue diversity using molecular markers, mainly from United States accessions, there is a lack of knowledge on the global diversity all over the world for this species. The objectives of this study were to evaluate the sub-structure of tall fescue world wide variability and to place French cultivars into this sub-structure. AFLPs (Amplified fragment length polymorphism) were chosen because they quickly deliver a high number of markers across the whole genome. One plant per accession from 37 ecotypes and 40 plants from six French cultivars were used. A total of 116 polymorphic fragments were scored from two primers combinations. Data analyses on the ecotypes revealed two highly distinct clusters. One cluster included three accessions from North Africa and Sicily and should correspond to Mediterranean tall fescue type. The second cluster included ecotypes from all over the world. This cluster could be sub-divided in three clusters. Cluster 1 included 27 accessions coming from all over the world except North Africa. This large cluster could come from an introduction of West European ecotypes in United States and then from this location introductions all over the world. Cluster 2 included 3 accessions from Morocco and Mauritania. Cluster 3 included 5 accessions from Germany, Yugoslavia, Russia and Turkey. Clusters 2 and 3 could come from different introductions of West European ecotypes in North West Africa and East Europe, respectively. Data analyses on French cultivars in comparison to ecotypes showed that the cultivars were all included in the large cluster of West European cluster 1.

P12

Analysis of Tunisian perennial ryegrass germplasm using simple sequence repeat primers

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Tunisia, like any other Mediterranean country has been recognized as rich source of genetic diversity for forage and pasture species. Perennial ryegrass (*Lolium perenne* L.) is one of the most important pasture grasses in Tunisia. It is an important cool-season bunchgrass, widely used in mixtures for pasture, lawns, hay and erosion control. This crop is of economic importance for forage production and turf. The present study portrays the achievement of the genetic polymorphism surveying and the establishment of an ecotype identification key on the base of simple sequence repeat data. Four spontaneous populations and one introduced cultivar (10 plants/population) were analysed using six microsatellite loci. A total of 28 alleles and 43 genotypes were revealed and permitted to evidence high degree of genetic diversity mainly explained at the inter group level. In addition, the microsatellite multilocus genotyping has permitted to unambiguously distinguish 48 well-defined ecotypes (a resolving power of 96%). Data are discussed in relation with the reliability of the used markers to rationally manage the conservation of this crop.

P13

DIVERSITY OF SIMPLE SEQUENCE REPEAT (SSR) MICROSATELLITE MARKERS IN TUNISIAN *Festuca arundinacea* Schreb. ACCESSIONS

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Tall fescue (*Festuca arundinacea* or *Lolium arundinaceum* Schreb.) is a cool-season forage grass that is widely grown throughout the temperate regions of the world. It is the most important perennial forage species in the northeast of Tunisia. The objective of this study was to characterize the local genetic diversity. Four spontaneous populations sampled from different eco geographical origins were exanimate using four microsatellite loci specific of perennial ryegrass. The derived genetic distances values suggested a high level of genetic diversity in the Tunisian germplasm. In addition, results have permitted to precise the correlation of molecular markers with agronomic characteristics and the identification of selected genotypes. Taking advantages of the designed procedures, this study portrays the opportunity of such methods to precise the genetic diversity organisation of tall fescue. Results are discussed in relation with a sustainable characterization of diversity to provide efficient markers suitable in order to establish breeding programs.

Keywords: *Festuca arundinacea* Schreb., genetic diversity, SSR

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O6

Evaluation and Utilization of Morphological Variation in a *Medicago truncatula* Core Collection

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Medicago truncatula is a model species for legume biology and has been used to develop tools for molecular genetics and genomics. Nested core collections have previously been identified using molecular markers (Zhang et al., 2009) representing the existing genetic diversity from the USDA germplasm collection. The practical value of nested core collections is that they allow implementation of efficient strategies to characterize phenotypic variation compared to random selection of accessions from the whole collection or using geographic stratification to select accessions for evaluation. The goals of this research were to efficiently use the *M. truncatula* core collections to characterize the natural existing variation associated with morphological traits. We assayed variation in pods (length, number of coils, direction of coiling, number of pods per raceme), leaves (morphology and pigmentation patterns), and roots (morphology and biomass) from accessions included in the nested core collections. Significant natural variation was identified for the morphological characteristics evaluated in pods, leaves, and roots. Accessions with contrasting phenotypes for multiple traits are currently being used to develop mapping populations. The *M. truncatula* core collections are publicly available and should enable researchers to efficiently evaluate genetic variation for additional traits of interest, further understand plant development, identify single nucleotide polymorphism (SNP) markers, select diverse genotypes to use in breeding programs, and conduct QTL, association, and comparative mapping studies to facilitate subsequent map-based cloning of desirable genes. The *M. truncatula* germplasm collection represents a source of genes that can be used to improve agricultural crops.

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O7

Collection and evaluation of Azerbaijan forage and crop genetic resources

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Abstract

A forage germplasm collection mission in Azerbaijan was conducted in 2004 by a team of scientists from SARDI, ICARDA and the Azerbaijan National Academy of Sciences Genetic Resources Institute.
Seed of 674 accessions were collected representing 37 genera and 129 different species, including 56 perennial and 73 annual species from a broad range of geographical regions.
Wild ecotypes of lucerne and its rhizobia were targeted in this collection mission, particularly *Medicago sativa subsp. caerulea*, which was collected at 40 diverse sites from mountains to deserts and beaches. Importantly this species was observed with roots actually growing in permanent fresh water. Soils at collection sites for *M.s.caerulea* ranged from clays to non-wetting sands, very wet and slightly saline areas through to very dispersive sodic clays. Soil pH ranged from 5.8 to 9.5 and the frequency of this plant decreased as sites became more acidic.
Trifolium tumens was the second target species of the expedition and was collected at 36 sites, quadrupling previous ex-situ conservation of this species. The range of adaptation and frequency was much greater than expected and the collection team are confident that increased drought and grazing tolerance has been collected above that which exists in current germplasm holdings.
Major forage grass species were *Dactylis glomerata* and *Lolium perenne*. Wild cereal relatives included seven named species of ancient and wild wheat (*Aegilops* and *Triticum*) and four species of barley (*Hordeum*) including the perennial species *H.bulbosum* and close relatives. Pulse and oil seed crops collected include *Brassica*, *Lens* and *Pisium*.
The SARDI Genetic Resource Centre has conducted characterisation, preliminary evaluation and seed multiplication activities on the perennial *Medicago* accessions collected from this trip. A brief report on the diversity of traits that confer herbage quality and production of this collection will be presented. This material has now entered regional field evaluation trials in SA, Tasmania and WA to identify drought tolerant perennials for Australian farming systems.

O8

Genetic diversity in a collection of lucerne populations evaluated by SSR markers

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Abstract
Structure of lucerne populations from the Mediterranean Basin is little described. Each population is adapted to pedoclimatic conditions but seed exchanges are frequent. We used seven microsatellite markers to evaluate within-population diversity and test for differenciation among populations. A set of 16 populations from France, Italy, Tunisia, Algeria, Morocco, Australia and the USA was analysed, each represented by 30 individuals. Number of alleles and expected heterozygozity were calculated to evaluate within-population diversity. F_{IS} was calculated to test departure of populations from panmictic equilibrium. Population structure was analysed by the calculation of F_{ST} index. The matrix of F_{ST} between pairs of populations was used to draw a dendrogram and define groups of related populations. Softwares taking into account the autotetraploidy of the species were used in all cases.

Number of alleles ranged from 12 to 37 for the seven markers. Number of alleles was similar for each population. Expected heterozygozity ranged from 0.673 (Coussouls) to 0.760 (Tamantit), indicating a large within-population diversity. One marker was excluded from the analyses because of presence of null alleles. Using the remaining six markers, the populations appeared to be at panmictic equilibrium. Global F_{ST} was significant but very low (0.016). It was similar to those calculated in other studies on lucerne populations (Flajoulot et al., 2005; Herrmann et al., 2008). F_{ST} between pairs of populations varied from 0 for the populations Sardi10 and African to 0.049 for the populations ABT805 and Gabes. Three groups were identified, one composed by only Gabes, a second group composed by Siriver, ABT805, Tamantit, Erfoud, Sardi10, Demnat203, African and a third group with the populations Ecotipo Siciliano, Prosementi, Mamuntanas, Ameristand, Melissa, Coussouls, Magali, Rich2.

A large within-population diversity is observed in Mediterranean populations of lucerne as in other populations. Differentiation among populations is limited, even if agronomic traits could show strong differences. This situation could be explained by the numerous gene flows among populations (through seed exchanges or pollen fluxes). Reproductive biology (allogamy) and genetics (autotetraploidy) favours the maintenance of large within-population diversity and limits the possibility of population differenciation for neutral markers. This apparent lack of differenciation is not related to agronomic traits that noticeably differ among populations.

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P14

Drought survival of some perennial grasses in Moroccan rainfed conditions: Agronomic traits

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Abstract

A four year experiment was carried out to evaluate perenniality and some adaptative responses to drought of *Dactylis glomerata*, *Festuca arundinacea* and *Phalaris aquatica*, within the multi-site activity of the EU-PERMED project. The trial held in Merchouch experimental INRA station at 68 km west of Rabat/Morocco, in a vertic deep silty clay soil (> 1m80 deep) was sown on 20 october 2005 to compare 16 grass accessions. Measurements included: sward establishment, dry matter production, average row cover, sward senescence and phenology. Plant emergence and establishment were good and regular for almost all cultivars. In all seasons, festuca yielded significantly higher than dactylis. The harsh conditions and summer droughts have affected significantly production and persistence of grasses, mostly dactylis cultivars which disappeared completely at the end of the third year except the summer dormant cultivar Kasbah. Four groups of grass cultivar have been distinguished according to their persistence under extreme drought conditions.

Key words: Perennial grasses, perenniality, summer dormancy

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P15

Drought survival of some perennial grasses in Moroccan rainfed conditions: Eco-physiological traits

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Abstract

A four year experiment was carried out to evaluate perenniality and some adaptative responses to drought of *Dactylis glomerata*, *Festuca arundinacea* and *Phalaris aquatica*, within the multi-site activity of the EU-PERMED project. The trial held in Merchouch experimental INRA station at 68 km west of Rabat/Morocco, in a vertic deep silty clay soil (> 1m80 deep) was sown on 20 october 2005 to compare 16 grass accessions. Measurements included: establishment, dry matter production, leaf area index, ratio green/senescent, sward senescence, water content in survival organs and water soluble carbohydrates.

The potential of production was important for tall fescue and phalaris compared to dactylis cultivars. Drought progression was different through varieties. Summer dormant dactylis, became totally senescent at onset of drought, while non dormant dactylis had more green tissue. Festuca cultivars had slightly slow senescence along summer and behave identically against drought progression. Senescence levels reached more than 87% at the middle of summer drought. Water content in survival organs decreased gradually under summer drought. Festuca cultivars had maintained higher levels at the end of summer (48%) than dactylis cultivars (39%), which did not show significant differences between varieties. Inversely, soluble water carbohydrates in survival organs increased with drought in the same pattern.

The harsh conditions and summer droughts have affected significantly production and persistence of grasses. Four groups of grass cultivar have been distinguished according to their persistence under extreme drought conditions.

Key words: Perennial grasses, perenniality, summer dormancy, sward senescence, water content and water soluble carbohydrates.

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P16

Perenniality and productivity of some spontaneous Algerian populations of two forage grass species (*Dactylis glomerata* L. et *Festuca arundinacea* Schreb.)[1]

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Abstract

Persistence is considered as a key parameter in the selection of forage grasses. It reflects the ability of plants to survive the harsh environmental conditions and other forms of stress during successive cycles. These cultures will be perfect for the southern countries, where animals suffer from lack of grazing over a long period of the year (Lemaire 19991). This work focuses on the study of evolution of this character in southern Mediterranean environment (Metidja, Algiers), where long periods of summer drought are the major constraint for perennial forage species. The plant material studied is composed of 10 spontaneous populations of *Dactylis glomerata* L. and 4 populations and one control variety of *Festuca arundinacea* Schreb. The results of the perenniality and dry matter production were observed on the crop seasons 2007 and 2008, corresponding to the 3rd and 4th growing year. Both years have been characterized by very early summer drought. The results yielded a classification of both species and populations within the same species on studied characters. For dry matter production, one cut has been registered for the 3rd season and two cuts for the 4th. Tall fescue varieties were most productive with 4 t DM / ha and 3.13 t DM / ha for cocksfoot varieties on the first cropping season and a total of 0.63 t DM / ha and 0.5 t DM / ha on the second season, respectively for the two species. The 4th year of study confirms that tall fescue is more resistant than cocksfoot to summer drought conditions. The means of species for survival plant, respectively for tall fescue and cocksfoot, are of 70.58% and 60.88% compared with the initial number of plant growth. Lelièvre et *al.* (2004) showed that the persistence of plants is directly related to summer dormancy.

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P17

Forage and seed yield components in four French landraces of grass pea (*Lathyrus sativus* L.)

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Abstract
One of the aims of a concerted research between the Institute of Field and Vegetable Crops and the Faculty of Agriculture in Novi Sad is the re-introduction of the legume species such as grass pea in the Serbian agriculture. Among the first accessions of a grass pea collection within the Annual Forage Legumes Collection (AFLCNS) in Novi Sad were four accessions, kindly donated from the Laboratoire d'Ecologie Moléculaire, IBEAS, University of Pau, France. All of these four are local landraces from southern France, with Le Cambou collected in Dordogne and Fléchou, Parranquet and Bon Encontre collected at Lot-et-Garonne. A small-plot trial was carried out in 2006, 2007 and 2008 at the Experimental Field of the Institute of Field and Vegetable Crops at Rimski Šančevi, including the four grass pea accessions and two identical parts of the trials, one for forage and another for seed. In both parts and in each year, all four accessions were sown in early March, with a sowing rate of 120 plants m^{-2}, with a plot size of 5 m^2 and three replicates. The plants in the first part were cut in the stage of full flowering, while in the second part the plants were harvested in the stage of full maturity of seeds in the oldest pods. In each accession there were evaluated main forage and yield components and the simple correlation coefficients between each pair of them were calculated. There was a significant variability of both forage and yield components at the levels of both 0.05 and 0.01. In all four accessions, green forage yield per plant was in the highest positive correlation with number of internodes per plant, while seed yield per plant was in the highest positive correlation with number of fertile nodes per plant.

P18

Domestication of new Mediterranean annual pasture legumes

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Abstract
Fifteen years ago subterranean clover (*Trifolium subterraneum* L.) and annual medics (*Medicago* spp.) dominated annual pasture legume sowings in the Mediterranean-like climate of southern Australia. Since then a number of sustainability and economic challenges to existing farming systems have emerged, exposing shortcomings in these species and a lack of legume biodiversity. A selection program, largely based in Western Australia, with testing sites across southern Australia, has responded to these challenges by domesticating new annual pasture legume species, native to the Mediterranean basin, to overcome the deficiencies in traditional species (Nichols *et al.* 2007). Six new species to agriculture have been commercialised (*Ornithopus sativus*, *Biserrula pelecinus*, *Trifolium glanduliferum*, *T. dasyurum*, *T. spumosum* and *Medicago sphaerocarpos*), while *Lotus ornithopodioides* and *Melilotus siculus* are under evaluation. Traits incorporated include deeper root systems, protection from false breaks (germination-inducing rainfall events followed by death from drought), a range of hardseed levels, acid-soil tolerant root nodule symbioses, tolerance to pests, diseases and salinity and provision of lower cost seed through ease of seed harvesting and processing. The contributions of genetic resources, rhizobiology, pasture ecology and agronomy, plant pathology, physiology, entomology, plant chemistry and animal science have been paramount to this success. Quantification of any anti-nutritional or toxic chemicals in the species and the conduct of grazing trials have also been essential to confirm safety to grazing animals, while assessments have also been conducted to ensure that environmental weed risks are minimised (Revell and Revell 2007). A farmer survey in Western Australia has shown widespread adoption of the new pasture legumes, and this trend is likely to increase due to the increasing cost of inorganic nitrogen, the need to combat herbicide-resistant crop weeds and improved livestock prices. Mixtures of these legumes allows for more robust pastures buffered against variable seasons, soils, pests, diseases and management decisions.

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P19

Screening Romanian alfalfa germplasm for salt and water stress

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Abstract

Abiotic stress conditions cause extensive losses to agricultural production worldwide (Bray et al., 2000). Climatic changes will conducted at sever drought conditions and to aridization of some important regions in Romania (ONU report, 2007). One of the most important drought and salinity strategies for alfalfa *(Medicago sativa)* breeding which could reduce the influence of those limiting factors is to increase the cultivar tolerance. The present paper reports the reactions of some Romanian alfalfa genotypes to salt and hydric stress. The aim was to elucidate some physiological and metabolic aspects of those stresses in order to establish screening criteria to facilitate the development of genotypes with high tolerance to field stress conditions.

Seeds of nine alfalfa genotypes were sown in Mitcherlich plots filled with a soil-sand mixture. The plant were grown in vegetation house under optimal condition up to before flowering, when for **hydric stress** variant the watering was reduced for 10 days, **salt stress** was impose on plants by adding *300 mM NaCl/l* and under **combined stress** the plants were treated with 300 *mM NaCl/l* one week before to reducing watering.

The alfalfa yield for all studied genotypes was significantly reduced under hydric and salt stress and stresses combination caused a reduction on fresh biomass, too. Salt stress significantly decreased biomass more 37 % while hydric stress more 73%. The effect of salt and water stress on yields are additive but not equal. Alfalfa respond to drought by decrease of leaves transpiration and between biomass accumulation and leaves transpiration under hydric and salt stress there are a linear relationships (r = 0.76*; r = 0.82*). Under optimal condition the proline content was very small but was obviously the high proline content under all stress treatments.

The negative effect of salinity and stress combined on alfalfa growth could be attributed to an osmotic effect. Osmotic stress inhibits water uptake from the soil and requires the plant to use energy and carbohydrate in synthesizing organic solutes to adjust its internal osmotic potential.

Yield loss results from closing stomata (as result the transpiration decrease) and from energy and carbohydrate use in osmoregulation. The leaves transpiration and biomass accumulation were correlated that indicated to be used as screening tools for drought and saline tolerance of alfalfa genotypes.

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P20

High-throughput detection of induced mutations and natural variation using KeyPoint™ technology

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Reverse genetics approaches rely on the detection of sequence alterations in target genes to identify allelic variants among mutant or natural populations. Current (pre-) screening methods such as TILLING and EcoTILLING are based on the detection of single base mismatches in heteroduplexes using endonucleases such as CEL 1. However, there are drawbacks in the use of endonucleases due to their relatively poor cleavage efficiency and exonuclease activity. Moreover, prescreening methods do not reveal information about the nature of sequence changes and their possible impact on gene function. We present KeyPoint™ technology, a high-throughput mutation/polymorphism discovery technique based on massive parallel sequencing of target genes amplified from mutant or natural populations. KeyPoint combines multi-dimensional pooling of large numbers of individual DNA samples and the use of sample identification tags ("sample barcoding") with next-generation sequencing technology. We show the power of KeyPoint by identifying mutants in the tomato eIF4E gene based on screening more than 3000 M2 families in a single GS FLX sequencing run, and discovery of haplotypes of tomato eIF4E gene by re-sequencing amplicons in a subset of 92 tomato lines from the EU-SOL core collection. We propose KeyPoint technology as a broadly applicable amplicon sequencing approach to screen mutant populations or germplasm collections for identification of (novel) allelic variation in a high-throughput fashion.

P21

The population genetic structure of diploid *Medicago sativa* L. germplasm

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Abstract

Although most cultivated alfalfa is a tetrasomic tetraploid, diploid germplasm are present naturally, but little evaluated. The simplicity of diploid genetic systems relative to tetrasomic tetraploids suggests more effort should be expended at the diploid level in order to understand the genetic basis of key traits. Our objective was to categorize the genetic diversity in diploid germplasm as a prelude to association mapping for yield and composition traits. A collection of 384 individual genotypes derived from 123 unimproved diploid accessions from the USDA National Plant Germplasm System was selected to represent the diploid *Medicago sativa-falcata* complex, including M*. sativa* subsp. *caerulea*, subsp. *falcata*, and subsp. *hemicycla*. The accessions were screened with 89 polymorphic SSR loci distributed throughout the genome in order to estimate genetic diversity, infer the genetic bases of current morphology-based taxonomy, and determine population structure. Phenotypic data was collected from field trials, including biomass yield, plant height, regrowth, and stem composition traits. High levels of variation were detected using SSR, with a mean of 18.4 alleles per locus and a mean heterozygosity across 89 loci of 0.485. A model-based clustering analysis of the genomic data identified two clearly discrete subpopulations, corresponding to the morphologically defined subspecies *falcata* and subspecies *caerulea*. The hybrid nature of subspecies *hemicycla* was confirmed based on its genome composition. Subsequent hierarchical population structures indicated that two distinct subpopulations exist within subspecies *caerulea* (northern vs. southern) and subspecies *falcata* (lowland vs. upland). When considering these five populations, about 81% of the estimated genetic variance resided within the populations leaving only 19% among populations. The biomass yield and agronomic trait data did not reveal any clustering among accessions, unlike the SSR results. This suggests that good alleles exist for different traits within the divergent germplasm, offering the opportunity to mine beneficial alleles from this material for cultivated alfalfa improvement. We are in the process of identifying SNP variants within genes associated with stem composition, including the lignin biosynthetic pathway. These markers will enable us to conduct association mapping to identify QTL for biomass yield and composition.

P22

Characterization and preliminary evaluation of *Hedysarum coronarium* L. ecotypes in Mediterranean environment

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Abstract
The changes of cropping systems in the last decades have caused a strong reduction of perennial forage genetic resources, especially for landraces of legume species and where the agriculture is more intensive.
The aim of our research was to characterize and preliminarily evaluate Italian genetic resources of sulla (*Hedysarum coronarium* L.).The trial was carried out in south Sardinia (Italy) during 2007-08 . Thirteen ecotypes of sulla representative of the main regions of cultivation (Sardina, Sicily, Tuscany, Abruzzo, Marche) and three control varieties ('Carmen', 'Grimaldi' and 'Sparacia') were evaluated in a trial designed as a randomized complete block with three replicates. In each plot (1.26 x 1.80 m) 70 plants were transplanted at the end of November, and on 40 plants the following variables were observed: winter mortality; growth habit in spring; time of beginning of flowering; dry matter yield; number of stems; leaf/stem ratio. These variables were analyzed using univariate and multivariate methods, and the results were used to identify genetic resources useful for local breeding of sulla.

P23

Phenotypic assessment of variability in tillering and early development in ryegrass (*Lolium spp.*)

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Tillering shows a huge variability within the genus Lolium. In order to exploit tillering characteristics in breeding programs, a systematic characterization of this trait at the between- and the within-species level is required. We carried out a detailed analysis of tillering patterns in a collection consisting of forage (mostly diploid, but also some tetraploids) and turf cultivars of *L. perenne,* forage cultivars of *L. multiflorum* and *L. x boucheanum* as well as some annual *L. temulentum* genotypes. The collection was also enriched with wild *L. perenne* populations. These wild populations are of particular relevance as potential sources of genetic diversity in branching genes that has not yet been exploited in breeding programs.

The parameters taken into account were: number of leaves on main stem, total number of tillers and re-growth capacity after cutting. The average number of leaves on main stem was lower in perennials as opposed to annuals. The total number of tillers exhibited a large variation among and within all investigated accessions. The turf grasses showed the most tillering, followed by wild populations, forage cultivars, and *L. temulentum*. Regeneration after cutting was relatively low in all accessions. Interestingly, other accessions than those with highest branching exhibited a high re-growth capacity. These observations suggest that different genetic mechanisms control tillering and re-growth. These results, based on the evaluation of individual plants, provide a first set of observations for understanding tillering behavior of individual genotypes in a sward.

P24

Cytogenetic mechanisms of species diversification in subfamily Poideae in Iran

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Abstract

Comparison of cytogenetic characteristics of 66 grass species belonging to 11 genera of subfamily Poideae was made considering ploidy level, chromosome pairing, heterozygote translocation, unreduced gamete formation and B-chromosomes. The genera studied possessed species with diploid, tetraploid, hexaploid and octaploid chromosome number while the genus *Melica* was an exception by possessing a very homogenous group of mainly diploid species. In the genera of *Aegilops*, *Avena, Alopecurus, Bromus*, *Catabrosa* and *Stipa* some of the polyploid species showed diplontic behavior possibly due to their allopolyploid nature or chromosome pairing controlling mechanisms, while some diploid and allopolyploid species like *Bromus brachystachys***,** *Festuca arundinacea*, *Secale cereale*, *Hordeum bulbosum*, etc. formed quadrivalents due to heterozygote translocations. The genera studied differed significantly in their chiasma frequency and distribution as well as chromosome pairing indicating their genetic distinctness. In some genera including *Avena* a sort of genetic mechanism on genetic recombination was noticed as the mean value of crossover among diploid species was higher compared to that of tetraploid and hexaploid species. Similarly tetraploids showed higher values compared to haploids. Unreduced gametes were formed in some of the species due to cytomixis or anaphase failure. Details of cytogenetic mechanisms leading to unreduced gamete formation and their frequency will be discussed. The occurrence of B-chromosomes and their effects on genetic recombination varied significantly among different species of each genus indicating mostly increasing the frequency of chiasma formation and genetic recombination.

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P26

Nordic timothy (*Phleum pratense* L.): Assessing genetic variation in a genebank collection

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Abstract
Timothy is the most important forage grass species in the Scandinavian countries, and therefore, a collaborative project 'Phenotypic and molecular characterisation of Nordic timothy' 2007-2010 including the following partners: Norwegian University of Life Sciences (Norway), University of Aarhus (Denmark), Agricultural University of Iceland, Graminor (Norway), Boreal Plant Breeding (Finland), NordGen (Sweden), Swedish University of Agricultural Sciences, and MTT Agrifood Research Finland. Finland's responsibility in the project involves analyzing genetic diversity in selected timothy accessions with SSRs (simple sequence repeat) and retrotransposon-based markers. Timothy is a hexaploid (2n=6x=42), cross-pollinating species which means high heterozygosity and also difficulties in genetic analyses. One hundred timothy accessions from the NordGen collection were selected based on geographical distribution and previous phenotyping data, and 20 individuals per population will be analyzed. The same populations have been in field trials in Norway, Sweden and Iceland for phenotypic characterisation. Fifteen timothy SSR markers were selected and optimized, and their selection was based on their easiness to score, repeatability and amplification from a single locus. The 15 microsatellites have also been optimized for multiplexing and pipetting robotics. REMAP (retrotransposon-microsatellite amplified polymorphism) analysis produces several markers (20-30) from one PCR. Fluorescein labelled primers will be used to enable scoring the results with a capillary sequencer. After collecting data from marker analyses, population structure and distribution of molecular variation will be studied and genetic distances between populations determined.

O2

Phylogenetics of genus *Dactylis* L. based on SSR marker analysis

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Abstract

Results of cytogenetic studies support classification of the genus *Dactylis* L. into a single species with more than 20 diploid, tetraploid and hexaploid subspecies (Hu and Timothy, 1971). Diploids are located in specialized habitats while tetraploids are the most common and generally distributed forms of *Dactylis*. However, the diploid and tetraploid plants could occasionally be found in the same site. Hexaploids have only been found in two localities. There are no modern taxonomic treatments which interprets all forms of *Dactylis* on the same basis. Based on recent studies, nuclear DNA content of the subspecies show tremendous variation even in the same ploidy levels (Tuna et al., 2007). The objective of this study was to investigate phylogenetic relationships among subspecies of the genus *Dactylis ba*sed on SSR marker analysis. Aproximatelly, 100 accessions representing more than 20 subspecies used in the study. Number of plants analysed for each accession changed between 3 and 5 plants. 10 primers out of 20 screened in the study generated scorable polymorphic bands. In this paper, an interpretation of results of the study will be presented and discussed.

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P27

Analysis of the clover (*Trifolium spp*) different species morphological traits

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Different species of clover *(T. pratense, T hybridum, T. repens, T. medium, T. montanum, T. alpestre, T. pannonicum, T. fragiferum. T. ambigum, T. ochroleucum)* were tested at the Lithuanian Institute of Agriculture in Dotnuva 2007 and 2008. Dry mater yield, leafs, stems and inflorescence weights, plant height, bunch diameter were evaluated.

T. pratense had the significantly highest DMY (226,12 g) of all investigated species, also it had high stem (121,74 g) small inflorescence weight (23,41 g) and large bunch diameter. *T. repens* and *T. medium* had significantly wide bunches (51,92 and 45,15 cm) and bigger leaf weight (74,25 and 69,3 g). *T. ochroleucum* plants were high (29,46 cm), bunches were wide and DMY was significantly high (21,53 g). *T. fragiferum* plants were the lowest (3,07 cm), their leaf weigh was significantly highest (93,85 g), stem weight significantly smallest (15,73 g.) of all investigated species. *T. alpestre* plants were stunted, bunch diameter was significantly smallest (16 cm), and inflorescence weight – significantly high (91,8 g). High variation of all parameters, except for DMY, was estimated in *T. hybridum*. Bunch diameter, DMY and leaf weight variation was low in *T. montanum* and *T. fragiferum*. *T. ochroleucum* stem height and leaf weight variation was average; variation of other parameters of this species was low. *T. repens* bunch diameter and DMY variation was average, variation of other parameters was high.

T. pratense and *T. ochroleucum* can be used for forage due to its' high dry matter yield. *T. fragiferum* can be used for installing turfs.

P28

The Czech core collection of *Trifolium repens* L.

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Altogether 32 characters were evaluated in the set of 161 accessions (varieties, newly bred varieties and wild forms collected in the nature) within world collection of the *Trifolium repens* L. stored in the Czech national gene bank. Thirty plants of each origin were planted on the field, ten of them were evaluated in the years 2007 and 2008. All the evaluated characters were included into the analyses. Missing values were replaced by mean value. Cluster analysis was performed in the software Statistica for Windows both for all the accessions together. Complete linkage method was used for clustering and Euclidean distance as the measure of distance.

Established core collection of the white clover consists of 41 origins from 17 countries. Czech Republic has 8 origins; United Kingdom has 5 origins; New Zealand, Netherlands and Denmark have 4 origins; France has 3 origins; Sweden and Poland 2 origins and Finland, USA, Germany, Japan, Ireland, Estonia, South African Republic, Latvia and Hungary have only one representative. Form *hollandicum* is in the collection 17 times, form *gigantem* 9 times, form *silvestre* 8 times and by 7 origins we did not find the form. Altogether, 39 subclusters for selection of representatives for the core collection were determined. Origins of the most widespread form *hollandicum*, are concentrated in 12 subclusters. Origins of the form *giganteum* are concentrated in 3 subclusters. Origins of form *sylvestre* are scattered through other subclusters.

O1

Towards an enhanced utilization of plant genetic resources in grass breeding by characterization and evaluation trials

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Abstract

The Malchow Satellite Collection of the IPK Genebank is holding a large grass collection with more than 10,000 accessions. Besides maintenance, characterization and evaluation of the most important grass species are the fundamental tasks of genebank work.
In recent years and in cooperation with universities, breeding companies and research institutions, we have performed several trials in this respect.
The most comprehensive analyses were performed in *Lolium perenne* L. (no. of accessions maintained at Malchow: more than 2,500), with a special focus on material from a collection trip to Romania. Primary evaluations of 455 ecotypes showed e.g. large variations in traits like heading date and growth type, partially outperforming standard varieties. Based on these results, individual plants of ecotypes of the same origin and with identical traits were lumped together to 85 subgroups and introduced as 85 additional accessions into the genebank. These were then subjected to secondary evaluations (green matter yield, crown rust susceptibility, content of endophytes). Here, 27 % of the accessions showed to be resistant to crown rust, and again, some accessions of the intermediate to late maturity group did as well as the standard varieties – or even better.
Similar trials were conducted with collection material from Bulgaria and Croatia, especially with regard to crown rust response and its correlation to the presence of endophytic fungi and to maturity group, respectively.
In summary, it is demonstrated that the collected ecotypes form a suitable basis for ryegrass breeding.

Ecological and population genetic concepts for creating new varieties?

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The agronomic value of grasslands tends to decrease over time, leading to the need for repeated ploughing and resowing, which cause long term environmental damage. In order to extend the time during which grasslands are productive, we must understand the causes for this decline. Changes in population genetic structure of agronomic grasses due to the interaction of selection, migration and drift may provide part of the answer.

Selection pressures can be mediated by abiotic factors (e.g., cultural practices, soil, climate) or biotic factors (e.g., competitors, diseases). Gene flow into sown grasslands may occur via pollen or seed from the surrounding landscape, and small effective population size may exacerbate genetic drift. Given that grasslands are composed of multiple species and cultivars, similar mechanisms may control their species composition. Different pedo-climatic conditions, different species and genetic grassland compositions and different cultural practices lead to different adaptations of grassland community.

Productivity in sown grasslands will be determined initially by the sown composition of species and genotypes within species. Productivity and resistance to invasion can be enhanced by complementary resource use among species or genotypes. Declines in productivity may arise if the traits that determine the initial success of species or genotypes are negatively correlated with traits that determine productivity over the long term. If so, taking measures to enhance the maintenance of diversity over time may extend the period of high productivity in sown grasslands, and developing cultivars or mixtures for which this is unlikely, are important future goals.

P29

Selection of spaced plants of perennial ryegrass in association with white clover

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Abstract
In conventional breeding programs of perennial ryegrass spaced plants are usually evaluated on bare soil with the application of mineral nitrogen fertilizer. In organic farming perennial ryegrass is mostly sown in association with white clover without any dressing of mineral N fertilizer. Will the selection under these organic conditions lead to varieties that are different from varieties obtained by a conventional breeding management?
In 2007 we installed 48 seedlings of each of 12 diploid and 9 tetraploid populations as spaced plants on bare soil under a conventional management with the application of 220 kg N/ha/year. 48 seedlings of each of the same populations were planted in a freshly sown stand of white clover without any N fertilizer application. Growth and crown rust infection of these plants were observed in 2008
Almost no crown rust occurred on the plants in the white clover sward. Although there were differences in competition and N availability the ranking of the growth scores of the diploid populations was nearly the same under both managements. The ranking of the tetraploid populations however was very different esp. for spring and summer growth. Plants under both managements were selected for half sib seed production in isolated polycrosses for further progeny testing.

P30

Determination of botanical composition in multispecific forage mixtures by near infrared spectroscopy (NIRS).

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Several methods to measure botanical composition of grasslands are described, such as visual scoring or manual separation. The most efficient method in which a large number of samples can be routinely analysed is the prediction by near infrared spectroscopy (NIRS). The objective of this study was to elaborate NIRS equations to predict the proportion of each species family (grass vs. legume) and also the proportion of each species in grass or grass-legume forage mixtures.
Samples were collected in multispecific sown swards ("real samples") and artificial samples were produced from samples collected in monospecific swards in spring or autumn in several years at Lusignan (France). The mixtures were composed by perennial ryegrass (Lp, *Lolium perenne*), cocksfoot (Dg, *Dactylis glomerata*), tall fescue (Fa, *Festuca arundinacea*) with or without white clover (Tr, *Trifolium repens*). Plant samples were dried and grounded, and NIRS spectra were collected on a NIRS Foss 6500 apparatus. Reflectance was measured for wave lengths between 1100 and 2500 nm. A partial mean square regression was employed. A total of 2930 artificial samples and 677 real samples was analysed.
The proportion of white clover in the mixtures was very well predicted, with a standard error of cross validation (SECV) of 1.7% and a R^2 of 1. Contrastingly, perennial ryegrass was the worsely predicted species with a SECV of 7.6% and a R^2 of 0.94. An external validation was realised on 80 real mixtures. Standard error of prediction (SEP) were 9.7, 8.7, 10.4 and 5.6% for Lp, Dg, Fa and Tr, respectively. An important bias was observed, but after correction, standard error of cross prediction was finally of 8.2, 5.9, 6.5 and 2.8%, respectively.
Prediction of Tr proportion was thus accurate. Similarly, Locher et al. (2005) obtained a SECV for the prediction of legume proportion in mixtures from 2.4 to 3.3%. Indeed, grass and legume samples differ for morphological and biochemical composition. The predictions of each grass species proportion were satisfactory, especially considering that their morphological and biochemical characteristics are close. It is the first time that grass species proportion is predicted by NIRS in forage mixtures.
This equation could be improved by including other samples from real mixtures grown in other locations. However, this equation can already be used to rapidly determine species composition in multispecific grasslands.

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P31

Investigating the competition for water and the depth of water extraction in plurispecific grasslands using the ^{18}O natural abundance

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Abstract

Even with a close control of their management, the botanical composition of simple mixture grasslands often exhibits large changes during their exploitation. This is due to the changes with seasons and years in the competition intensity for light, water and minerals. In particular, during the 4 to 6 years of the grass crop, the partitioning of the yield between the various species depends on their respective ability to use the water resources of the soil during the dry periods of the year. The soil water contributes to satisfy the plant demand, depending on root depth and location of water in soil. The dominated species should have shallower roots due to the plant response to shade and their demand in water should be less than in dominating species, bringing about all together a shallower depth of water extraction (DWE) in the dominated species. Using soil humidity measurements repeated in time at different depth, the DWE of a species is easy to measure in pure swards. That is irrelevant in mixed swards because (i) roots of all species can be found in all horizons and (ii) the relationship between root density, soil humidity and root extraction is complex. Furthermore, we have to date no way of identifying precisely enough the grass roots of different species in soils, even using the newest and most promising methodologies. To date the use of natural abundance of ^{18}O is the only way to precisely compare the DWE of plants sharing the same ground (Durand et al 2007). It is based on the occurrence of a gradient in the soil water isotopic composition, due to the evaporation – induced enrichment of the surface ^{18}O abundance in water. This natural phenomenon occurs when the soil water profile is regular and after a few days (typically a week) without any rainfalls. The methodology is commonly used in vegetation studies but it was never used in mixed temporary grasslands.

The work reported here describes the results obtained in a mixture of *Dactylis glomerata/Lolium perenne/Festuca arundinacea* grown in dense sward and believed to exhibit various abilities to exploit soil resources (Durand et al 11997). Pure stands were also followed and compared to the mixed crop. The results showed that when the soil profile exhibited a monotonous gradient of natural ^{18}O abundance in water, the ranking of the DWE of three species was possible and explained the differences in plant water status observed in summer. The impact of such differences on the competition for water and equilibrium between species is discussed.

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P32

Botanical and genetic change in grass-clover based systems

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Abstract
Since the use of nitrogen fertilisers has been limited in Flanders, swards based on grass-clover mixtures regain importance in grassland production. The population structure in these swards develops in response to abiotic and biotic stresses. In this study we analysed the botanical and the genetic change in grass-clover mixtures.
We have screened 15 varieties of white clover and 15 varieties of red clover by AFLP markers. We selected 5 varieties of each of the species which have narrow diversities within the variety and high genetic distances between the varieties. On the 1st of June 2005 we have sown a field experiment with these clover varieties in association with perennial ryegrass (Table 1). The plots were mown three to four times during the growing seasons of 2006, 2007 and 2008.
The botanical composition was analysed at the last cut of each year. In the plots with the red clover mixtures the proportion of the grass was low even in the sowing year. The percentage white clover was low in the mixtures with both legumes in 2005, 2006 and 2007 (respectively 12%, 16% and 14%). Since red clover is not persistent white clover was expanding in these plots in 2008 and the proportion of white clover was increasing to 50% in the autumn of 2008.
In August 2008 we took samples on the plots of type A and B to test the genetic shift of one white or red clover variety growing in association with perennial ryegrass. These samples will be analysed by AFLP markers and compared with the originally sown seed lots.

Table 1. Different types of grass clover mixtures used to analyse the botanical and genetic change. (RC: red clover variety; WC: white clover variety)

type	number of plots	description
A	5	each of the RC
B	5	each of the WC
C	1	mixture of the 5 RC
D	1	mixture of the 5 WC
E	1	mixture of the 5 RC and the 5 WC
F	5	each of the RC with a mixture of the 5 WC
G	5	each of the WC with a mixture of the 5 RC

P33

Plasticity *versus* selection in morphological evolution of populations. Case study of *Lolium perenne* L. mini-swards.

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Abstract

Sown meadows are highly diverse including both species and genetic diversity. Yield of sown meadows decreases over years leading to the necessity of ploughing and re-sowing new meadows which is not without environmental impacts. So, a challenge is to understand how meadows evolved in order to be able to increase their yield persistency. This evolution could come from both plants selection and plasticity. Indeed, the decrease of yield in a meadow could be due to a selection for the most persistent genotypes which would be the less productive and/or an adaptation to defoliation of all the genotypes by limiting their size (Sibly, 1995 ; Hazard and Ghesquiere, 1997). The objective of the study is to quantify the part of both selection and plasticity in a perennial ryegrass mini-sward evolution under two evolutive pressures defined by two defoliation frequencies. The population used is a pseudo F2 from a cross between one forage and one turf genotypes consisting of 240 genotypes. The part of selection and plasticity will be evaluated over three generations of selection pressures. This will be realised using morphological traits measures including plant height, leaf length, dry matter and number of tillers and using fitness value evaluation via the reproductive effort and mortality. Genetic evolution will be followed using “neutral” and “selected” molecular markers identified in QTL analyses on adaptive traits.
The first results from morphological data after the first year of selection pressures highlight plasticity in leaf length and dry matter between two rhythms of defoliation and significant differences between genotypes.

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P34

Adaptation of a Mediterranean orchardgrass population to the south central USA

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Abstract

Typical US cultivars of orchardgrass (*Dactylis glomerata*) are poorly adapted to the southern Great Plains (Hopkins, 2005). Summer dormancy in other cool season grasses has been associated with improved persistence in this region (Malinowski et al., 2005), and may have value in orchardgrass. Thus, our objective was to determine the adaptation of a Mediterranean orchardgrass population selected for persistence in the south central USA. The population NFOG 101 was developed by selecting Plant Introductions originating from Morocco for two cycles of persistence under heavy grazing in the south central USA. Seeded sward plots to determine persistence were grown in Oklahoma and Texas, and a space planted nursery was used to gather morphological data. Stands of one year old clipped plots in Oklahoma averaged as follows: NFOG 101 (98%); Benchmark Plus (57%); Persist (52%); Paiute (37%). In an Oklahoma grazing tolerance trial, stands averaged 97% for NFOG 101 vs. 69% for Benchmark Plus. In Texas, stands of NFOG 101 were 76% compared to 66% for Benchmark Plus. Heading date of NFOG 101 in Oklahoma is about one and four days earlier, and average plant height about 20 and 10 cm shorter, than Benchmark Plus and Potomac, respectively. In the southern Great Plains, NFOG 101 acts as a semi summer dormant population. Results to date indicate that NFOG 101 has superior grazing tolerance and persistence compared to US cultivars, and could provide an option for those wanting to grow orchardgrass in the south central USA. Cultivar release of NFOG 101 is anticipated in the coming years.

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P35

The contribution of hybrid ryegrass (*Lolium x hybridum* Hausskn.) to dry matter yield in a forage seed mixture.

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Abstract

In UK agriculture, forage grasses are invariably grown in mixtures composed of different varieties and species, usually with white clover. It is generally thought that having several varieties and/or species in a sward confers enhanced flexibility. Some decades ago grass seed mixtures could comprise many varied components which would later be reduced to 6 species in the Cockle Park mixture (Lazenby, 1981) but examination of modern seed catalogues shows the majority of mixtures are comprised of various combinations of ryegrasses with or without clover.

A common mixture type comprises mainly perennial ryegrasses of both intermediate and late flowering types to provide yield and ground cover respectively. Very often a hybrid ryegrass will be added to provide additional dry matter yield at the main silage cut. The aim of this study was to provide information on the contribution of the hybrid ryegrass to the yielding ability of such a sward over three harvest years. The mixture chosen was a typical silage ley mixture comprising 23% hybrid ryegrass, 31% intermediate flowering perennial ryegrass, 23% late flowering perennial ryegrass and a 23% of a second late flowering perennial ryegrass. A further 10 seed mixtures were used with the contribution of hybrid ryegrass ranging from 0% to 37% of the sward.

A set of 3m x 1m plots were established in 2005 and harvested over 3 years using a 7 cut combined management. A regression analysis was carried out for the yields in each harvest year against the percentage of hybrid in the sward. Dry matter yield responded linearly to the percentage hybrid in each harvest year (2006 $p<0.01$; 2007 $p<0.01$; 2008 $p<0.05$) confirming that the hybrid ryegrass had a significant effect on the yield of the mixture even in the third harvest year. No significant differences were observed in the slopes of the lines ($p = 0.188$) indicating a similar response to the percentage of hybrid ryegrass in the mixture in all 3 harvest years.

Further experimentation is required to determine if this response is reproducible under differing environmental conditions.

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P36

Availability of selected perennial ryegrass varieties for use in grass/legume mixtures

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Abstract
Perennial ryegrass is often used for the establishment or oversowing of highly productive pastures. Since entry of the Czech Republic into the European Union in 2004 the European Common Catalogue of Varieties of Agricultural Crop Species has become effective in this country. However, the end-use properties of most of the varieties given in the European Common Catalogue have not been properly tested in Czech soil and climatic conditions and so there is a risk that farmers will use cheap seeds of varieties which will not have adequate performance, disease resistance and persistence. Therefore, the forage productivity, persistence, disease resistance and competitive ability of selected perennial ryegrass (*Lolium perenne*) varieties were investigated in small-plot trials under contrasting soil and climatic conditions at different locations in the Czech Republic. The productivity of varieties was tested at three different sites: Troubsko (270 m a.s.l.), Zubří (356 m.a.s.l.) and Vatín (540 m a.s.l.). Ryegrass varieties were tested in monoculture and in grass/legume mixtures with 50% proportion of tested variety. The trials showed the differences in availability selected perennial ryegrass varieties for use in different soil-climatic areas. The most plastic variety, that showing good results in all tested sites, was diploid cv. Aberelan. The best varieties in mixtures were achieved in diploid cultivars AberElan, AberDart, Olaf, Respect and the tetraploid cultivars Kertak, Jaspis and Alligator. The results were statistically tested using the ANOVA method and post hoc testing by Tukey.
The health of varieties of perennial ryegrass was tested predominantly by long-term snow cover and subsequent infection with snow mould (*Typhula incarnata*). The varieties most infected by this disease were Bravo, Premium and Respect. These varieties, alone amongst the tested cultivars, were also infected with fungi of the genus *Neotyphodium* spp. In summer and in autumn ryegrass varieties were also infected with leaf blotches which also infected the above mentioned varieties most

Key words: perennial ryegrass, variety, competitiveness

O4

Characterisation of variation in condensed tannin levels and persistence in *Lotus* spp.

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Abstract

Lotus corniculatus (bird's-foot trefoil) and *L. uliginosus* syn. *L. pedunculatus* (greater bird's- foot trefoil) are relatively minor perennial forage legume species within UK grassland. The varieties of both *Lotus* species that are commercially available have not been bred for UK conditions and tend to lack persistence in mixed swards (Hopkins et al., 1996). However both species have important characteristics with potential benefits for grassland agriculture. Their herbage contains proanthocyanidins, also known as condensed tannins (CTs), which help to reduce bloat, have anthelminthic properties and can protect protein in the rumen (Waghorn et al., 1992) while reducing the rate of protein degradation and nitrogenous losses to the environment. The levels of CTs in the herbage of *Lotus* species are critical as they can have beneficial or detrimental effects on ruminant livestock, depending on their concentration, which is influenced by a number of environmental and developmental factors. We have begun a programme of research to quantify the variation in CT levels and persistence within a number of varieties and selection lines as the first step in the identification of material which can be used in a crossing programme aimed at the development of varieties. We have used a high throughput method of quantifying the level of CTs in herbage of these *Lotus* species (Marshall et al., 2008) which has revealed significant variation in CT content within and between varieties. A CT content of up to 35mg/g dry DM was common among the 21 varieties analysed with some plants showing values of 70mg/g DM. One variety had a CT content of 150 mg/g DM, which is at a level that could have a negative effect on animal performance. Significant variation in plant persistence was also found with a rhizomatous line (Highgrove) exhibiting high levels of persistence. Current studies are aimed at crossing the persistent lines with those with appropriate CT levels and testing stability of CT levels and persistence of this material in different environments.

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O5

Festuca/*Lolium* specific markers for surveying genetic changes in Grasslands

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Abstract

Interaction between climate and genetic variability conditions any breeding program and occupies a large part in managing and conservation of biodiversity. For grasslands, understanding and modelling climate changes impact, over seasons and years, on their diversity and their agronomical value, is still a challenge for geneticists, eco-physiologists and ecologists. In this aim, *Festulolium* hybrids are quite valuable experimental plant material as they provide to selective forces relevant genetic variability in respect to traits such as cold or drought tolerance. They are also characterised by a high Linkage Disequilibrium that enables surveying genetic changes at the genome scale without requiring numerous molecular markers. However, highly species-specific markers are needed to associate genetic changes with possible effects of selection. Furthermore, *Festulolium* are tetraploid, which may lead to biased allele frequency because of complex genotypes. We report hereafter a screening of markers for genetic survey of *Festulolium* among 47 public STS and EST-SSR markers (Lem and Lallemand, 2003; Saha et al, 2004; Studer et al, 2008).

Test sample included extracted DNA of 33 and 36 *F. arundinacea var. glaucescens* (*Fg*) and *L. multiflorum* (*Lm*) respectively, in addition to progenitors used for developing newly registered *Festulolium* cultivars of *L. multiflorum* x *F. glaucescens*. Mean amplification rate was 0.73 and 0.80 for *Fg* and *Lm* (resp.) with 2 and 6 markers amplifying only one parental DNA (*Fg* and *Lm* resp.). Overall, 377 alleles were numbered with 2.02 *Festuca* alleles, 3.76 *Lolium* alleles and 1.19 common alleles per locus, on average. Specificity indices at each marker were computed from allele frequency in parental species as the rate of individuals in a theoretical hybrid population, assuming tetraploid inheritance at equilibrium, to which an unambiguous genotype could be assigned. 9 markers presented a specificity index superior to 0.9. No relationship was found between marker specificity and origin (STS/EST) or species (*L. perenne*/*F. arundinacea*) from which primer pairs were defined.

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O3

Plant architecture in ryegrass – an alternative route to more persistent perennial ryegrass varieties

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The architecture of plants is determined by complex interactions among genetic and environmental factors. It has been demonstrated that architectural changes can affect profoundly the yield potential and the quality of crops. We can also anticipate that in grassland swards, the presence of genotypes with improved tillering capacity will improve persistence. Although a wealth of knowledge exists in model plants, the genetic control of plant architecture remains poorly understood in most crop species. Today, the importance of plant architecture as a yield component is widely accepted, but the lack of readily applicable knowledge impedes a wider use of its potential for the improvement of yield and persistence in fodder grasses.

In *Lolium perenne*, knowledge needs to be generated at two different levels. First, the ideal branching phenotype needs to be delineated and the relationship of branching with other relevant traits, such as heading date and fodder yield and quality, needs to be established. Second, the genetic regulation of branching needs to be unravelled: which genes are important, what is their allelic diversity, and how can this genetic knowledge be applied in breeding and crop production. We are currently developing a translational approach to implement knowledge from model plants, such as *Arabidopsis* and tomato, in ryegrass. Genes with a clear function in branching will be investigated for their allelic diversity in *L. perenne* and putative correlations of particular alleles of those genes with tillering will be established through association genetics. A collection of *L. perenne* genotypes is being built and thoroughly phenotyped for the application of association genetics. First year observations of branching responses show an extensive variation in tillering and re-growth responses within and between cultivars and natural populations.

P37

Forage production of grasslands composed by one, two or three varieties of perennial ryegrass

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Abstract

Increase in specific diversity in grasslands was shown to have a positive impact on biomass production. This was mainly due to an increased diversity for functional traits (Hector et al, 1999 ; Roscher et al., 2007). However, even within a species, a large genetic diversity is available, for functional traits, such as heading date. Sown grasslands may be composed by a mixture of varieties coming from a single species (Humphreys and O'Kiely, 2006). The present study aimed at evaluating the effect of an increased genetic diversity on forage production of perennial ryegrass (*Lolium perenne*), the increased genetic diversity being achieved by mixtures of varieties.

Mixtures of varieties of similar or different heading dates were sown in 2003 in a micro-plot experiment conducted at INRA of Lusignan (France) with three blocks. Nine varieties grown in pure stand and 10 mixtures of two or three varieties were analysed. Two defoliation regimes were applied (fast or slow), under high or low nitrogen fertilisation. During five years, dry matter production was determined in each cut.

Considering plots composed by one, two or three varieties of the same heading date, no difference in annual forage production was noticed. Increased genetic diversity within a heading date group provided nor advantage neither disadvantage. The effect of heading date was highly significant on forage production: late varieties always producing less than intermediate or early varieties in this experiment. Mixtures of varieties of different heading dates had an intermediate forage production and never produced more than the highest yielding variety. Forage production was not improved by the mixture of varieties of different heading dates. However, this type of mixture can be a way to secure forage production under conditions that could favour either early or late varieties.

In addition to these results, distribution of forage production along seasons will be further analysed in this experiment. Consequences on quality traits will also be evaluated.

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Genetic gain in agronomic value of forage crops and turf: a review.

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Abstract.

Plant breeding has shown to be an effective process to design and develop varieties to serve the requirements of end users. However, in that respect grasses are very different from other species due to there perennial characteristic and the possibility to produce mixtures of species and varieties. Besides that there are many traits that are genetically negatively correlated so the varieties are compromises to suit the customers. In this presentation an overview will be given of available literature on quantitative characteristics like dry matter yield, nitrogen recovery or nutritional value, and the results will be compared with genetic gains in other crops based on recent quantitative-genetics tools. Besides that examples of genetic improvements for many other traits will be given as disease resistance to invertebrate pests and fungal, bacterial and viral diseases and abiotic stresses.
For the turfgrasses a substantial improvement in important agronomic traits has been obtained in the last 30 years. This improvement will be illustrated using data from official lists and literature. Improvements has in particularly been related to traits like sod density, fineness of leafs and tolerance to wear – whereas improvements in respect to diseases resistance like snow mould (*Fusarium nivale)* , red threat (*Laetisarium fuciformis*) and abiotic stress like drought have been more restricted due to low heritability of the traits. In order to meet the requirements to a world with more emphasis on low input which already is seen in some countries, focus in the breeding for the future will be on the more complex traits like drought and disease resistance than it has been in the past. Future research is proposed to focus even more on grass root development in order to allow for development for efficient breeding tools since good and efficient roots are crucial under low input.
A very important issue, the importance of good and stable seed yields, for the economic feasibility of grass products will be discussed.
Finally the potential of genomic selection approaches will briefly addressed.

Challenges for DUS/VCU Testing in Europe and Implications for Breeding Progress

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A consequence of the multipurpose function of turf and forage grasses and legumes is that providing definitive assessments of Value for Cultivation and Use (VCU) is extremely complex. A finely mowed golf green and a roughly cut roadside verge require very different turf attributes. Forage evaluation must account for diverse on-farm uses and that the end point is not the forage but a ruminant product. Consequently, there are many different VCU testing systems across Europe. Distinctness, Uniformity and Stability (DUS) testing is more internationally harmonised but no less complex, as allogamous species express ranges in their discriminating characteristics, giving high GxE sensitivity, complex statistics and no uniform qualitative characteristics to group and reduce reference collection numbers.

The challenges for testers are multiple. Advances in genomics and metabolomics and development of rapid NIRS analyses will provide new breeding innovations that require evaluation. European environmental policies promote reduced carbon footprints and will increasingly require VCU standards that improve fertilizer efficiencies and lower ruminant excretions. In DUS systems, unfavourable candidate-control ratios continue to inflate test fees and workloads. The implications of these factors plus retracting public funding across Europe will be considered, including options for greater bilateral DUS agreements and the possibilities for taking better consideration of the climatic zones at the European level.

P38

Evaluation of yield potential, genetic variances and correlation for nine promising cultivars of alfalfa under the New Valley environment

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ABSTRACT

In the period of 2004-2006, a field trail was conducted at the New Valley Res. Station to evaluate the yield potential and genetic variances among alfalfa cultivars; a new Synthetic (Wady Syn.), four promising populations ($Serw_1$, $Serw_2$, Nitrogen fixation and salt tolerant), three commercial varieties (Siwa, $Ismailia_1$ and $Ismailia_{94}$) and a local cultivar (Wady). Twenty cuts were obtained during 2005 and 2006. The combined analysis of variance over two years indicated that Wady Syn. population ranked first for fresh and dry yields (71 t fed^{-1} and 18.9 t fed^{-1}) and other studied traits significantly different from other tested cultivars. The commercial variety $Ismailia_{94}$ ranked second (66.55 and 16.85 t fed^{-1}). Regarding plant height, tillers and leaf to stem ratio (LSR), Wady Syn. recorded the highest values (48.23 cm, 416.67/m^2 and 47.58 % significant from $Ismailia_{94}$ which recorded 45.6 cm, 362.33 m^2 and 43.33 %., respectively. Significant positive correlation among either fresh forage yield or dry f orage yield and other traits. The values of genotypic coefficient of variation for fresh and dry forage yields revealed relative variations among the tested cultivars which were less influenced by environment. The environmental variation ranged from 4.7% to 8.6% and the genetic advance ranged from 3.9% to 14.5%.

Keywords: Alfalfa, Yield, Variability, correlation.

Abbreviation: fed. = 4200 m^2

P39

Modelling adaptive responses across agricultural environments as a prerequisite for identifying adaptive traits and plant ideotypes

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Abstract
Modelling cultivar yield responses to agricultural environments by additive main effects and multiplicative interaction (AMMI), factorial regression or joint regression is useful for understanding genotype × environment interaction patterns, simplifying the targeting of cultivars, improving the predictive ability of the results, and helping breeding programs in defining selection strategies, adaptive traits and plant ideotypes (Annicchiarico, 2002). A major objective of the EU-funded project PERMED (Improvement of native perennial forage plants for sustainability of Mediterranean farming systems) is defining selection strategies and adapted plant types for lucerne, sulla, tall fescue and cocksfoot in the Mediterranean region. The current study provides preliminary information on adaptive responses of cocksfoot, sulla and lucerne cultivars across locations featuring different drought-stress levels. Tolerance to drought had a major impact on cultivar adaptive responses (e.g. sulla: Annicchiarico et al., 2008). Site-specific responses of cocksfoot material proved largely affected by its level of summer dormancy. Lucerne cultivars which showed contrasting adaptation pattern were chosen for inclusion in a physiological study in which shoot and root traits were evaluated in metal containers (55 cm × 12 cm × 75 cm deep) under different drought-stress levels. These artificial environments were able to reproduce the cultivar adaptive responses across agricultural environments, unlike another experiment carried out in Tunisia using 30 cm-deep pots. The experiment in metal containers suggested the occurrence of different mechanisms for enduring drought.

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O12

Application of molecular markers for variety protection in ryegrass (*Lolium perenne*)

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Abstract

Based on morphological characteristics, new ryegrass varieties have to be new, clearly distinct (D) from known varieties, sufficiently uniform (U), and stable (S) to be eligible to be registered at the National List or granted plant variety protection. Molecular markers offer the potential to deliver precise information on DUS assessment at lower costs. The overall objective of this project is to examine the potential use of molecular markers (specifically SSRs) for the description of genetic variation in ryegrass varieties to evaluate their possible use in DUS examination.

In 2004, only one diploid perennial ryegrass variety was entered in the official DUS trial for the second and final year of testing. Based on results from the first year of DUS testing, 16 reference varieties, four control varieties and the candidate variety were selected for the project (i.e., in total 21 varieties x 60 genotypes). The reference varieties were similar to the candidate variety based on standardised morphological characterisation within the first year of the DUS trial. For each variety, plant material was freeze-dried and DNA was extracted from 60 individual genotypes. 18 SSR markers were selected based on their genome distribution, reproducibility, polymorphic information content and ease of scoring.

Estimation of genetic distances, cluster analysis and Mantel test were performed with the NTSYS-PC software package. Preliminary results based on a subset of the data showed that the dendrograms obtained from UPGMA cluster analyses on the basis of genetic distance estimates resulted, in most cases, in a separation of all of the varieties. Furthermore, significant correlation was found between the molecular data and the morphological data, indicating that SSR markers can be used for variety identification.

P40

Ecological and biometrical characteristics of galega cultivars

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Abstract

The new Lithuanian fodder galega (*Galega orientalis* Lam.) high–yielding cultivars 'Vidmantai', 'Laukiai' and 'Melsviai' were bred during 1986–2005 at the Research Station of the Lithuanian Agricultural University applying group and individual selection of progeny from the wild populations. In 2000–2001 the cultivars 'Vidmantai', 'Laukiai' and 'Melsviai' were registered in Lithuania. These cultivars have been sent to Estonia for DUS testing in 2007. A good adaptability to Lithuanian agro–climatical conditions are established for bred galega cultivars, which guarantee early, heavy, protein–rich yield and high resistance to phytopathogens and pests. Average data of 5 years of the competitive trials of dry matter yield rated from 12.1 ('Vidmantai') up to 13.9 ('Melsviai') t ha^{-1}. Stable yield of seeds (0.58–0.62 t ha^{-1}) was established. The mean protein content rated 218.3–243.5 g kg^{-1}.

Key words: galega, eco–bio–testing, cultivar

P41

Natural stress water effect on water use efficiency and behaviour of sixteen cultivars of perennial alfalfa (*Medicago sativa* L.) in semi-arid region (H'Madna, Algeria)[2]

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Abstrat

The alfalfa in addition to its survival is a forage crop which has several advantages in the sustainability of the agricultural system (Volaire and Norton, 2006). Its deep roots (up to 10 m) give it the ability to adapt and resist drought (Mauriès M., 1994). For this purpose, sixteen cultivar of alfalfa (*Medicago sativa* L.), from several sources are being tested at H'Madna in Bas-Chéliff under a semi-arid climate. Both trials are conducted under two different water regimes (rainfied, ETM). The cultivars capacity evaluation is made through the study of the water use efficiency of the plant (WUE) and the dry matter yield (t ha^{-1}). The results of the 3rd and 4th year of production on the subject of an analysis of variance and comparison tests of averages (Newman-Keuls and Fisher LSD). For the 3rd year (Rainfield trial), cultivar Rich2 is the most efficient (2.25 kg $DM.m^{-3}$). As against the African cultivar is the least efficient (1.4 kg $DM.m^{-3}$). The cultivar Mamuntanas gives the best production with 8.34 t $ha^{-1}$and the least productive Tamantit with 4.50 t ha^{-1}. For the irrigated trial, Rich2 is the least efficient (1.56 Kg MS/m^3), Ameristand 801S most efficient (2.44 kg $DM.m^{-3}$). For the 4th year (Rainfield trial), cultivar Sardi10 is the most efficient (0.69 Kg MS/m^3) and more productive (1.26 t MS/ha); Magali 1 is the least efficient (0.17 kg $DM.m^{-3}$) and least productive (0.33 t MS/ha). For the irrigated trial, cultivar Ameristand 801S is the most efficient (0.88 kg $DM.m^{-3}$) and most productive (8.89 t ha^{-1}); the cultivar Magali 1 is the least efficient (0.39 kg $DM.m^{-3}$) and least productive (3.82 t ha^{-1}). In rainfied regime, cultivar Mamuntanas is the perennial with Trec% to 32.90%, Gabès-2355 with a Trec% to 62.90% is the least sustainable. In the irrigated (ETM), Magali is the least sustainable with Trec% to 43.12%. By against Demnat 203 is more durable with a Trec% to 4.15%.

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O10

The proteins involved in cold acclimation of perennial ryegrass (*Lolium perenne* L.)

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Abstract

Perennial ryegrass (*Lolium perenne* L.) is one of the most important forage grass species of the temperate region. It is characterized by good productivity, forage quality and relatively high winter hardiness. Similarly to all the plants which can survive winter, *L. perenne* has the ability to increase frost tolerance after exposure to low but non-freezing temperatures (this adaptive process is known as cold acclimation, CA). Cold acclimation influences several physiological and biochemical pathways in plants reprogramming cell metabolism and gene expression. Unfortunately, the details concerning CA and resistance to frost are not well identified and understood for forage grasses. To recognize the mechanisms of cold acclimation in *L. perenne*, the complex research was initiated, including: (1) the selection of two plant individuals with distinct levels of frost tolerance, (2) the comparison of protein expression profiles after different time of CA between the most and the least frost tolerant plant using 2-D electrophoresis, (3) the identification of proteins which were differentially accumulated at least at one time point of CA between the selected plants using mass spectrometry. In this work we present results of plant selection and preliminary results of the proteome screening performed on 2-D maps.

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P42

Evaluation of drought tolerance variability in Mediterranean alfalfa cultivars in the field under Moroccan conditions

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SUMMARY

Sixteen alfalfa cultivars originating from the Mediterranean basin are tested in an experimental station in Morocco, located in the semi arid bioclimatic stage. This research was conducted in PREMED project during 2006 to 2008 and aimed to evaluate the adaptation of cultivars to drought stress. The aspects relating to perenniality and water use efficiency (WUE) will be correlated with yields, row cover, leaf senescence and summer dormancy. The trial was conducted under two irrigation treatments. The first treatment was normally irrigated, and in the second treatment water deficit was applied by stopping the irrigation during 9 weeks in summers. Results showed that application of water stress during the summer has significantly reduced the biomass of all cultivars. This reduction varied between 48% and 65% according to cultivars in comparison with a normal irrigated treatment. The difference between cultivars for the biomass was significant only in stressed treatment (P = 0.006 with 15 d.f). Some cultivars showed interesting yield potential, mainly Ameristand, Sardi10, Siriver, Gabes-2355, Rich2, Erfoud1 and Dem203. The mean rate of leaf senescence of cultivars was 69% with no significant difference between cultivars. The row cover estimated in the end of summer of the third year varied between 20% and 40% according to the cultivars. Cultivars: Gabes-2355, Dem203, Rich2 and Erfoud1 showed less number of plants disappeared under water stress. Therefore, those cultivars can provide a possibility for an alternative use by local farmers in the region.

Key words: alfalfa, drought, irrigation, biomass, perenniality

O22

Field resistance of *Festuca rubra* varieties to red thread (*Laetisaria fuciformis*)

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Abstract

As the progress in breeding for resistance to some typical turf diseases is not very clear (Feuerstein and Roulund, 1994), a field collection of 6 varieties of sheep fescue (*Festuca ovina*) and 35 varieties of red fescue (*Festuca rubra sensu lato*) was used to assess the incidence of red thread (*Laetisaria fucifirmis*) in the years 2005 -2007. Whereas in the first cutting year (2005) disease incidence was very low and did not provide enough information about intervarietal differences, in the years 2006 - 2007 the level of disease in the collection of red fescue varieties was very high. Sheep fescue varieties had high resistance and red thread incidence was not recorded. In the collection of *F. trichophylla* varieties, Barpearl, Baroyal and Viktorka had high resistance (area damaged did not exceed 5 %). They differ significantly from other varieties. Among the varieties of *F. rubra* ssp. *rubra* there was none with higher resistance (damage was 95 %). In the collection of *F. nigrescens* the varieties Barborka and Citera had high resistance (damage was less than 10 %) and differ significantly from other varieties of this species. There was no connection observed between disease incidence and experimental treatments (fertilizer application, cutting frequency). A hypothesis was confirmed that with low levels of fertilizer application there is considerable expression of this disease, especially in older stands (Metz et al., 2000), and the selection of donors of resistance is optimal.

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O9

Change in agronomic performance of *Lolium perenne* and *Lolium multiflorum* varieties in the past 40 years based on data from Belgian VCU trials

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Abstract

By using data of Belgian trials for Value of Cultivation and Use (1963-2007) the change in agronomic performance of ryegrass varieties was quantified. Data included 144 *Lolium perenne* and 69 *Lolium multiflorum* candidate varieties, plus 'Vigor' and 'Lemtal', two varieties which were always included in the VCU trials. Since the genetics of 'Vigor' and 'Lemtal' have remained identical to those in 1963, these were used as constant standards to measure improvements of new varieties. Dry matter yield (DMY) of 'Vigor' and 'Lemtal' varied annually but did not show a progressive change, indicating that cultural changes in VCU trials of ryegrasses were small. By expressing DMY, persistency and rust resistance of the candidate varieties relative to 'Vigor' and 'Lemtal', the change in agronomic performance due to breeding was determined. The relative DMY increased by 0.3% annually for both ryegrasses. The rather small improvement in DMY was due to a low scope for altering the harvest index of grasses, costly and complicated breeding work, no reliable and economical system to hybridize inbreds, and the lower financial investment due to a lower sales of grass seed (Wilkins and Humphreys, 2003). Persistency was markedly improved with 0.5% annually. The main increase in rust resistance occurred after the 1990s, when it increased with 3.5% and 3.8% annually for *L. perenne* and *L. multiflorum* varieties, respectively. This was due to (1) more attention for rust resistance in the 1990s since limitations to N fertilization had increased problems of rust infection and (2) a more efficient screening of rust resistance (e.g. controlled artificial infections; Reheul and Ghesquière, 1996). The agronomic performance of early *L. perenne* varieties improved less compared to late and intermediate varieties since the latter were more interesting for the farmer (used for both grazing and mowing), and hence they achieved more attention in breeding.

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O11

Studies on several romanian agroecotypes of birdsfoot trefoil (otus corniculatus L.), with special reference to their productivity and forage quality

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The studies carried out at University of Agricultural Sciences and Veterinary Medicine Timisoara revealed the presence of several Romanian and foreign agroecotypes of birdsfoot trefoil with outstanding qualities from the standpoint productivity and forage quality. The content of cyanogenic compounds was also investigated, a high variability of this latter characteristic being observed both between and within the birsdfoot trefoil agroecotypes evaluated. In essence the paper present following: average dry matter yields in different agroecotypes, variability of raw protein content and variability of the content of cyanogenic compounds.

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P44

Results regarding to promovation in crop of Phalaris arundinacea cultivars for production of forage and biomass

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The experiment was carried out at Grassland Research Institute - Brasov Romania, referring the behaviour of different cultivars of *Phalaris arundinacea*, from which the Premier variety certified.

It was study the forage quality, chemical composition at *Phalaris arundinacea* comparative with *Festuca arundinacea* and *Dactylis glomerata* in years with different climatic characteristics (2006 - 2008). In this study for the first time in our country were carried out the studies more detailed on improvement of *Phalaris arundinacea* for introduction in crop as forage and biomass. It is showed that the first Romanian cultivar, Premier assured a DM production with 40 % superior to those cultivars of *Dactylis glomerata* – Regent and *Festuca arundinacea* – Adela. In the drought years, 2006 -2007 the same species – *Phalaris arundinacea* which is resistant to moisture excess had the highest production, however, *Dactylis glomerata* and *Festuca arundinacea* are recognized as having the best resistant to drought. Also, the quality of *Phalaris arundinacea* is enough of high some synthetics are qualitative superior to of *Dactylis glomerata* and *Festuca arundinacea* cultivars. In the future of climate global heating it is appreciated that *Phalaris arundinacea* will be spread in the crop due to its adaptable characteristics to extreme conditions: large moisture and drought. It was proposed that the first harvesting to be used as biomass – fuel and the followings as forage for animals.

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P44

Impact of four decades of breeding on molecular differentiation between forage and turf cultivars of *Lolium perenne*

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Abstract (399)

Life-history traits make genetic differentiation in grass species relatively low; using isozyme loci Charmet et al (1993) estimated F_{ST} to be only of 0.054 within a collection of 550 French ecotypes of *Lolium perenne*. However, breeding may also change genetic structure in crops. From dominant molecular polymorphism (*RAPDs*), Bolaric et al (2005) found that the between-component of genetic distance variance slightly increased from 29 % among ecotypes to 33 % among cultivars. Herein, we aimed to evaluate the impact of breeding in perennial ryegrass with special emphasis on divergence of forage *vs* turf cultivars and by using co-dominant markers (*SSRs*). The genotype sampling was identical to that used by Sampoux et al (this meeting) for assessing genetic progress among 54 diploid ryegrass cultivars registered on European national lists from 1965 to 2004, *i.e.* 31 turf and 18 forage cultivars, 5 undifferentiated old cultivars and 7 ecotypes originating from various European climatic areas. Overall, about 2300 individuals (34 individuals/cultivar x locus on average) were genotyped at 10 *SSR* loci isolated from expressed *DNA* sequences and sampling at least one of each of the 7 linkage groups of *Lolium perenne*. F_{ST} between ecotypes was found to be 0.061, in good agreement with the estimation of Charmet et al (1993), whereas F_{ST} between cultivars was nearly doubled to 0.103 (forage) and 0.114 (turf). F_{ST} between forage and turf types was only 0.012 with quite large mean heterozygosity (0.55). A consistent classification using Euclidian distance computed from frequency of 107 alleles and *UPGMA* clustering resulted in 6 clusters and only two outlying single cultivars (*Idole* and *Aberavon*) accounting for 37 % of total variance. Four clusters included only turf cultivars and another one, only forage cultivars. When plotted onto the 2 first axis of a factorial discriminant analysis, the six former clusters surrounded the seventh cluster mixed with old cultivars of both type and ecotypes from Belgium, French Brittany and central part of France as this one had been the major source of diversity used for breeding. In conclusion, it is suggested that breeding structured genetic diversity within *Lolium perenne* as a pool of partially inter-related cultivars keeping genetic drift of the whole species at a particularly low level. Likely, breeding programmes in the future should partition more closely genetic variability in *Lolium perenne* to gain further efficiency.

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P45

Behaviour of five Sulla (*Hedysarum coronarium* L.) cultivars in a sub humid region (Algiers)[3]

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Abstract

The Sulla is a biennial legume in the Mediterranean region with agricultural and livestock interests. In the North of Algeria, some *Hedysarum* spontaneous species are used for feeding; in the South, other species are also used for the production of honey (Abdelguerfi-Berrekia et al., 1991). Whereas in Setif ITGC station, the soil and climatic conditions are semi-arid, *Hedysarum coronarium* showed interesting potential for production (Abdelguerfi-Laouar et al., 2002). For this, we studied, on two years, the behaviour of five cultivars of Sulla conducted in a Subhumid climate (Algiers). The trial allowed differences between cultivars on the early flowering and production (in green matter and dry matter). The dry matter yield values ranging from 3.09 $t.ha^{-1}$ to 8.34 $t.ha^{-1}$. For the two years the behaviour was different with the best production in the second year. The cultivars of *Hedysarum cornarium* are very promising in our region; they have good adaptation and production.

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P46

Study of behaviour of sixteen perennial alfalfa cultivars (*Medicago sativa* L.) under two water regimes in a sub humid region (Mitidja, Algeria)[4]

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Abstract

In Mediterranean region, perennial alfalfa is widely used in livestock feed. This species has a large spectrum of adaptation (Abdelguerfi and Laouar, 2002). It is grown in dry or irrigated conditions, across the Maghreb. To study the behaviour of sixteen alfalfa cultivars, an experiment, on two years (2006-2008) with two trials one under irrigation (ETM), and the other under rainfed condition, was carried out in Mitidja (Subhumid). Under rainfed conditions the cultivars give accumulated dry matter which varies from 5.45 to 10.19 t ha^{-1} and from 2.93 to 8 t ha^{-1} respectively for the two cropping seasons 2006-07 and 2007-08 corresponding to the 3rd and 4th year of production. For irrigated trail, the values vary from 7.32 to 13.73 t ha^{-1} and from 7.74 to 16.23 t ha^{-1} respectively for the 3rd and 4th year. Differences in earliness and persistence are observed between cultivars; some cultivars are distinguished by a good and fairly stable production from one year to another, it's the case of the cultivars Mamuntanas. This cultivar gives, under both water regimes, high yields and good water valorisation with values of water use efficiency (WUE) of about 2.9 and 3.4 kg $DM.m^{-3}$ under irrigation respectively for the two years and 2.3 kg $DM.m^{-3}$ under rainfed (same value for both years). During the first two test years this cultivar gives already a high WUE ranging from 2 to 3.5 kg $DM.m^{-3}$ (Khelifi et al., 2008).

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P47

Root development and drought tolerance in Mediterranean types of *Medicago sativa* L.

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Abstract

Alfalfa is widely used as rain-fed forage crop under sub-humid and semi-arid climates. In Mediterranean areas where 3 or 4 months may be totally dry, drought tolerance (summer survival) depends on slow extraction of deep soil water reserve across summer to maintain roots, crown and basal buds hydrated. The ability of young alfalfa plants to establish rapidly a deep taproot and to maintain deep root growth during the onset of summer drought, are key traits for optimal establishment of crops in their first year and summer survival. The objective of this study was to analyse variations of root growth (dry matter, depth), root/shoot ratios, and root growth maintenance during onset of drought in a range of six Mediterranean *M. sativa* L. accessions. Two groups of cultivars (cvs) were studied : (i) four erected high yielding cvs: **Cuf-101**, a US cv of oasis type, having low fall dormancy (mark 10) ; **Melissa** and **Cinna**, two French Mediterranean cvs (fall dormancy marks of 7 and 6 respectively); **Coussouls**, a French cv selected from dry Provence areas, semi-erected, with fine stems, less yielding than Cinna and deeper fall-winter dormancy (mark 5); (ii) a second group of two genetic pools selected by INRA France from wild *M. sativa* 'mielgas' from dry regions of Spain, with deep fall dormancy: **ES-96** (prostrate) and **Gazon** (creeping with rhizomes).

Plants were sown in autumn in a glasshouse. In spring (2 May), stems were cut at 5 cm, roots at 15 cm depth, and plants were transplanted in deep pots of plastic tubes (depth 2.6 m, section 23.7 cm²) filled with the same quantity (9 500 $\pm$ 20 g) of dried sandy soil, at 2 plants per tube and 24 tubes per cv, under a rainout shelter in Montpellier (France). After an irrigated cycle for plant establishment (root depth around 1 m), a cut was done on 28th June (day d_{-2}). Four tubes per cv were kept under optimal irrigation as controls until d_{39}. On 30th June (day d_0), water supply was totally stopped in 20 tubes per cv, which were sampled at d_8, d_{23}, $d_{39\text{-}s}$, d_{79} (the irrigated at $d_{39\text{-}i}$). At each date, a set of 4 tubes per cv was opened to record: aerial dry matter (DMA); root depth (RD); root biomass (DMR) shared in taproot+crown (DMT) and fine roots of diameter <1 mm, (DMF), all DM values in g DM per pot.

Under optimal water supply, aerial growth rates and DMA were much higher (approximately +50%) in classical erected varieties compared to mielgas. Root biomass DMR show the same important differences, even slightly higher. But taproots elongation rates and depth were not significantly different (1.92 m and 1.77 m respectively, not significantly different, in the control at $d_{39\text{-}i}$.). DMT represented 54% of DMR in classical cvs, but 64% in mielgas. In droughted treatment, stem elongation stopped totally between d_{15} and d_{18} with the same difference in height and DMA than under irrigation (around +50% for erected cvs). During the same period, daily root elongation rates were constant and similar for all cvs during ten days and then decreased slowly; they were still important (around 1.5 cm.d^{-1}) when aerial growth stopped totally (d_{15} to d_{18}). Root growth continued a long time after the stop of shoot growth, in average +45 d and +0.29 cm depth in classical varieties, and much more (+60 d and+0.57 cm) in mielgas. Average final root depths (d_{79}) reached 1.61 m in classical cvs, but were deeper (1.76 m) in mielgas. These results show that wild "mielgas" originating from dry regions of Spain concentrate root growth in taproots at depth and maintain deep root growth much better than classical varieties at the onset of summer drought. These root traits highly linked to summer drought tolerance and survival should be conserved in selection.

Key-words : alfalfa, mielgas types, root growth, root/shoot growth, drought tolerance.

P48

Variability and correlative relationships of important traits of red clover (*Trifolium pratense* L.) half-sib progenies

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The level of variability and correlative relationships of breeding material for the most important agronomic traits is very important in red clover breeding programs. Two diploid red clover populations, which showed good or satisfactory results in agro-ecological conditions in Serbia were used in experiment. During two years in NC I design, 60 plants from every of 45 half-sib progenies from both red clover population, got from a procedure for cross-pollinated plants, suggested by Comstock and Robinson (1949) have been researched. Obtained results mostly depended on genotype and the year of research. The population 1 had higher mean values for researched traits in both years of the research. In population 2, in the both years of the research have been found higher values of genetic and phenotypic coefficients of variation for all the researched traits, except for total sugar content. Values of genetic and phenotypic coefficients of correlation in population 2 were lower than population 1. The highest values of positive genetic coefficient correlation for both populations have been recorded for a dry matter yield and plant height (0,726** and 0,443**) and for crude cellulose content (0,754** and 0,351*). In both of investigated populations have been found average strong and significant correlations between number of tiller per plant and total sugar content and crude cellulose content. Negative values of genetic and phenotypic coefficient of correlation for both researched populations founded for dry matter yield per plant and crude protein content and also between crude protein and crude cellulose content.

P49

Performance of some perennial grass varieties under semi arid growth conditions[5].

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Abstract

Within farming systems, perennial grasslands provide numerous positive environmental effects such as improvement of soil structure, erosion decrease, control of nitrate leaching and maintenance of biodiversity. In order to develop grassland crops with improved drought tolerance and enhanced water use efficiency, 14 cultivars of which 7 of cocksfoot (*Dactylis glomerata* L) and 7 of tall fescue (*Festuca arundinacea* Schreb.) made the object of a survey of agronomic behaviour and drought tolerance evaluation in the semi arid region of Sétif during the 2007-08 cropping season, corresponding to the third year of production. The measured traits have focused on accumulated dry matter (DM), water use efficiency (WUE), plant height (PHT), relative growth rate (RGR) sward persistence (PER) and earliness (DHE). The study has revealed the existence of some variability for certain criteria; it allowed us to distinguish varieties characterized by a good dry matter yield and good water use efficiency. The varieties of tall fescue are most productive, very early and show a good drought tolerance, while Cocksfoot varieties have an average yield, an acceptable resistance to drought and late heading

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P50

Breeding and selection on a multifoliolate line originated from the Italian alfalfa (*Medicago sativa* L.) cultivar Minerva

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Abstract
The occurrence of multifoliolate leaves (four or more leaflets instead of three) in alfalfa may be an occasional phenomenon or a recurrent trait. Inheritable multifoliolate leaf characteristic has a potential agronomic interest, because it may result in a higher leaf/stem ratio and therefore in a higher protein and digestible fiber content in comparison with the wild-type trifoliate leaf trait.
Inheritable sources of the multifoliolate leaf characteristic were found in U.S.A. populations and transferred into several multifoliolate varieties, which showed an appreciable percentage of multifoliolate leaves. Multifoliolate varieties bred in the United States were marketed in Italy since 1993, but they only partially exhibit the multifoliolate trait, especially in summer. In addition they are in general more winter dormant than the average Italian cultivars.
Società Produttori Sementi started in 2003 a program to study the higher frequency of multifoliolate leaves in the variety Minerva (bred by Società Produttori Sementi S.p.A. and registered in 2004) compared with other Italian cultivars. A novel source of inheritable multifoliolate leaf characteristic was recognized, which was transmitted through three half-sib generations. The genotypes obtained showed up to 50% multifoliolate leaves even in the hot dry summer season. The plants belonging to the first half-sib generation were identified one by one and progressively selected both on phenotype and by progeny test. Object of this work is a synthesis of four years observations, based on visual assessment of multifoliolate leaves percentage and of vegetative and reproductive fitness of the spaced plants.
The selection led to the start of a mother plant field showing regular expression of multifoliolate leaves and positive agronomic traits. A cross between this multifoliolate line and another novel multifoliolate genotype, encountered in the Italian cultivar La Bella Campagnola, gave origin to the cultivar Miranda, submitted for registration in Italy.

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P51

Genetic diversity within and among alfalfa varieties for some traits

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Increasing variability in selection material could be achieved by introducing distance alfalfa varieties, which could be new source of diversity. The aim of this investigation was to determine the productivity and morphological traits of different varieties from USA in comparation with domestic varieties and populations in order to find the genotypes with good agronomical traits, suitable for improvement of domestic varieties. In eight cuts in second and third year of utillization dry matter yield, plant height, number of steam, steam thickness, leaf- steam ratio, regrow after cutting of alfalfa varieties have been examined. Results showed a significant differences between varieties in almost all investigated traits. Mean values and intervals of variability indicate high genetic potential of evaluated varieties for all examined traits. The highest variability among varieties was obtained for dry matter yield (CV 14%), number of steam (CV10.8%) steam thickness (CV 10%) and regrow after cutting. The lower coefficient of variation (about 10 %) was noticed for other traits. The high coefficient of variation observed for almost all examined traits indicated a significant genetic variability within varieties. Individual plants within varieties were showed higher variability for dry matter yield, number of steam and steam thickness (CV 45-50%), than for other traits (CV 15-30%). Cluster analysis for investigated traits, calculated by the Ward method, using the Euclid distance, showed diversity among investigated alfalfa varieties. Wide genetic variability of agronomic traits, especially for regrow after cutting, steam number and steam thickness in alfalfa cultivars provides a good basis for the improvement of domestic varieties and creation of new cultivars with great potential for high and quality yields of forage and hay.

P52

Genetic improvement in rye-grass (*Lolium perenne*) from turf and forage breeding over the four past decades

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Abstract

The most direct way to assess the efficiency of plant breeding in a species is to compare within a common experiment the performances of cultivars released over a time span covering several decades. In order to assess the efficiency of breeding in rye-grass (*Lolium perenne*), we tested the agronomic performances of a set of diploid cultivars including 32 turf cultivars and 22 forage cultivars released on European national lists from 1965 to 2004. Seven ecotypes, originating from the main European climatic areas were also tested. Turf and forage trials were performed at six and four locations, respectively. All turf and forage cultivars and ecotypes were furthermore assessed at two locations in spaced plant trials and in seed production trials.

Multivariate analyses highlighted two main independent dimensions of variation among cultivars and ecotypes: (1) a first dimension relating to morphology, vegetative biomass, and turf aesthetic quality, (2) a second dimension relating to disease resistance (rusts, red thread). Linear regressions fitting agronomic traits to the year of release enabled to test the genetic improvement in turf and forage breeding through time. The genetic improvement in turf breeding was highly significant (p value of regression slope < 0.001) for most target traits (general aesthetic quality, disease resistance, wear tolerance, summer aspect, persistency). The rate of improvement of the general aesthetic aspect equalled 58% of the standard deviation of the cultivar set range per ten years. In forage breeding, the genetic improvement was highly significant (p value < 0.001) for autumn dry matter yield, rust resistance, and persistency, and was significant (p value < 0.05) for summer dry matter yield. The rate of improvement of annual dry matter yield reached 0.29 tons per ten years, a value similar to that estimated by Camlin (1997) and Tabel and Allerit (2005). Forage breeding was additionally associated with a highly significant decrease of lignin and crude protein content, and with a highly significant increase of soluble carbohydrate content. No significant change in grain production was noted either in forage or turf breeding, but conversely a large range of seed production was pointed out in both ancient and recent cultivars.

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O21

The EUCARPIA multi-site rust evaluation – results 2007

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Abstract
The EUCARPIA rust evaluation trials were established in 2007 for the third time. The same 33 perennial and 18 Italian ryegrass cultivars were tested as in the first and second trial of 2001 and 2004, respectively (Boller et al., 2003; Schubiger et al., 2007). In addition, the Italian ryegrass cultivars Crema and Gosia and the perennial ryegrass cultivar Maja were included. The trials were sown at 28 sites in 12 countries of Europe. Twenty one sites were the same as in the first and second trial.
Crown rust (*Puccinia coronata* f. sp. *lolii*) was again the most frequently observed rust on both ryegrass species. Variation in resistance to crown rust among cultivars was significant at 16 sites for perennial and 22 sites for Italian ryegrass. Stem rust (*Puccinia graminis* f. sp. *graminicola*) occurred mainly on perennial ryegrass. There was a significant difference in mean stem rust scores among cultivars at 12 sites.
The new cultivar Gosia and Tarandus showed the highest level of resistance of all the Italian ryegrass cultivars tested. Bocage, Gwendal and Lacerta were the most crown rust resistant perennial ryegrass cultivars. The ranking of the mean crown rust susceptibility of the cultivars was highly correlated with the corresponding ranking of cultivars in the 2001 and 2004 trials, respectively. This was true for both perennial and Italian ryegrass.

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P53

Dry matter production and nutritive value of perennial ryegrass cultivars collection

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Perennial ryegrass (*Lolium perenne* L.) is one of the most important perennial cool-season forage grasses. It is a high productive grass with the highest nutritive value among forage grasses, adapted for high defoliation frequency and grazing. Breeding of perennial grasses cultivar and their improvement is often difficult, laborious and time consuming process. Market demands direct interest of perennial ryegrass breeders is focused on production of cultivars with high, stabile yield and good quality of dry matter, which differ in maturity and resistance to drought, frost and other stressful conditions of the environment. According that, initial breeding material must be heterogeneous and with range of different genotypes, either cultivars or wild populations.

In this article perennial ryegrass cultivar collection has been investigated at the beginning of breeding process. The collection was consisted of 20 cultivars which originated from Europe and USA and cultivar K 11 from Serbia. The trial was sown on experimental field of the Institute for forage crops in completely randomized block designed with 2m^2 plots in three replications. Within a two-year period time of tillering, crop height in first and regeneration in second cut, annual yield and nutritive value of dry matter (crude protein, NDF, ADF, hemicelluloses and lignin) were investigated. The data were analysed by ANOVA on the basis of two-year mean values and cluster analysis was made by the Ward method, using the Euclid distances.

Tillering of cultivars ranged from 14. May for earliest (Switzerland) to 29. May for latest cultivars (Irish). Plant height in first cut was between 60.7 and 98cm and up to 51.7cm in second re-growth. Best annual dry matter yield achieved cultivars Mara (14.28 t.ha^{-1}), K-11 (13.93 t.ha^{-1}) and Respect (12.98 t.ha^{-1}), all being medium-late varieties. All parameters of nutritive value showed great level of variability.

P54

Variability and correlation between seed yield, seed quality and its components in alfalfa

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Alfalfa (*Medicago sativa* L.) is a very important and widespread perennial forage crop in Serbia. Besides dry matter yield and quality of hay, seed yield is also important traits in determining the effective distribution of alfalfa varieties to farmers. The objective of this investigation is to determined genetic variability and correlation for seed yield, seed quality and its components in alfalfa varieties. During three years, three cultivars of alfalfa from Serbia (NS Slavica, NS Medijana, and Zaječarska 83) and French cultivar Europe were observed in field trials in Zajecar, which is situated in very dry region of Eastern Serbia. Seed yield components (seed yield, plant height, number of plants per m^2, number of secondary branches) and seed quality (germination, dormant seed, weight of 1000 seeds) of alfalfa were investigated. In year of sowing, the highest variability is obtained for seed yield (CV=56,6%) and percent of dormant seed (CV=19,0%), while others characteristics showed low variability. The lowest variability was obtained for seed germination (CV=0,2%). In the second and third years of utilization the similar results were obtained. The highest variability was obtained for dormant seed (CV=14,7%), while variability for seed yield was much lower then in year of sowing (CV=11,0%). Other investigated characteristics were expressed low variability. The lowest variability was expressed in seed germination (CV=0,4%), too. High, positive correlation dependence between seed yield and plant height (r=0,910) was obtained. Seed germination (r=0,893) and dormant seed percent (r=0,873) were in high correlation with yield, too. Number of secondary shoots showed mean correlation coefficient (r=0,499). Between seed yield and 1000 seed weight high negative correlation was existed (r=-0,791). Seed yield and number of plants per m^2 showed negative correlation dependence, too (r=-0,574).

P55

Dry matter yield and quality of lucerne genotypes

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Abstract
Lucerne (*Medicago sativa* L.) is the principal forage crop in the world, not only of high yields of forage, but also for high nutritive value. The interest in wild has been remained (despite of progressive genetic engineering) due to needs for a greater range of environmental adaptation, better yield and quality characters in the plants and a wide base to solve problems of resistance to disease and pests.
Twenty-four entries of lucerne were studied in field experiment at Jõgeva Plant Breeding Institute (58°45'NL, 26°24'EL, the yearly air temperature 4.4°C, precipitation 655 mm) during 2003-2006. Among them were 11 varieties of different geographic origin and 13 local populations. Lucerne was sown in July 2003 without a cover crop with 4 replications, randomized block design, plot size 6m^2, seeding rate of 20 kg ha^{-1} of 100 % germinating seeds. Winterhardiness, dry matter, share of leaves in the yield, crude protein, neutral detergent fiber, and acid detergent fiber were measured. Forage was cut three times in the utilisation years. Forage yield variation and distribution of the yield during the growing season between the varieties and local populations was large.

P56

Influence of forage species, cultivar and cut on lipid metabolism during the ensiling process

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Abstract

Due to the link between human health and FA composition of the diet, fatty acid (FA) composition of ruminant products is receiving increasing interest. In ruminant nutrition forages are an important source of FA. However, it seems that lipid metabolism during storage of the forage can be of importance. This can be concluded from studies where a lower milk unsaturated FA (UFA) content is found when conserved forages are fed instead of fresh (Whiting, et al., 2004). Generally, lipid metabolism in silages consists of lipolysis, which is a release of FA. In the rumen lipolysis is followed by biohydrogenation, which is a saturation of UFA. The latter is very extensive in the rumen, leading to a high proportion of saturated FA (SFA) in the end product. However, SFA are generally considered unhealthy, whereas UFA, which are mostly present in forages, are generally healthy. Reducing lipolysis in silages could lead to lower biohydrogenation in the rumen and thus to a higher transfer of UFA to the end product. It has been shown that lipolysis in silages of red clover can be inhibited by red clover polyphenol oxidase (PPO) (Lee, et al., 2008) and in white clover silages possibly by highly present saponines (Lourenço, et al., 2005). In this work, lipolysis in silages of one ryegrass, three red clover and three white clover cultivars was studied over three different cuts throughout 2007. Generally, clover silages showed a lower *in silo* lipolysis than ryegrass. In red clover this could be due to the lipid protecting properties of PPO. However, *in silo* lipolysis differences between red clover cultivars were not correlated with measured PPO activity. The role of saponines in the lower lipolysis in white clover silages is subject of further study. An effect of cut on *in silo* lipid metabolism was also found.

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P57

Variability of the rumen escape protein and fatty acid composition of grass and clover species and cultivars

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Abstract
Increase of rumen escape protein (REP) and higher content of poly-unsaturated fatty acids (PUFA) in forage is getting more interest. In general, protein of grasses and clovers is extensively degraded in the rumen, resulting in a low nitrogen use efficiency by cattle and a high burden on the environment. PUFA are known to have many positive effects on animal and human health, but are also highly degraded in the rumen. In this work, fast, reliable and cheap methods were developed to predict REP and C18:3 content using dried, ground and stored samples of grasses and clovers. Prediction of REP was possible using a regression curve and robust NIRS calibrations for cellulase digestibility and ADF, as well as the determination of moisture. Evaluation of C18:3 content was also possible by NIRS calibration. Samples from plots (3 replicates, 5 cuts, 2 N fertilizer levels) of perennial and Italian ryegrass, timothy, orchard grass, meadow fescue, tall fescue, red clover and white clover were analysed using these methods. Variation for REP and C18:3 content was present between and within most grass species. Italian ryegrass and orchard grass presented the highest average REP while perennial ryegrass had the lowest one. For C18:3, timothy showed on average the highest content while Italian ryegrass had the lowest one. There were no significant differences in C18:3 content within the fescue species, neither differences in REP within the tall fescue. The clover results still need to be evaluated. Results of individual plants of different cultivars of grasses (perennial ryegrass and tall fescue) and clovers (red and white) will also be presented.

O20

Resistance of red clover to broad spectrum of Sclerotinia trifoliorum

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Abstract

Sclerotinia trifoliorum causes clover rot, a disease of major importance for red clover in northern and western Europe. This disease is difficult to control and resistant red clover varieties have yet to be developed.
In this research we first plan to investigate the genetic diversity of the pathogen *S. trifoliorum*, using AFLP on fungal isolates of many European countries. The next step is the development of a bio-test to screen red clover plants for their resistance against clover rot. This bio-test should be easy in use, since we aim to screen a large number of plants and fungal strains. The third step in this research is evaluating the fungal strains for their virulence and evaluating a broad collection of red clover varieties for their resistance against *S. trifoliorum*. We plan to evaluate about 100 red clover varieties (cultivars and wild varieties), including many varieties from the USDA red clover gene bank core collection. From each variety about 100 plants will be evaluated, since there often is a large variability within red clover varieties. Resistant plants may lead to the development of a new resistant cultivar. The last step in the research is to investigate the heritability of clover rot resistance in red clover. Therefore we will make crosses between resistant and susceptible plants. If the resistance is determined by multiple genes, a QTL analysis will be started.

Different isolates of *Sclerotinia trifoliorum* have been collected from different European countries. Different artificial media were tested for their ability to induce mycelial growth and the production of multiple big sclerotia in the fungus. A culture method optimal for DNA extraction has been established. DNA is currently being extracted from each isolate to perform AFLP studies. First experiments indicate that the largest sclerotia were formed on bread with red clover infusion. Sclerotia have been produced from each fungal isolate and the induction of ascospore production has been started. Produced ascospores will be used to construct the bio-test.

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P58

The effect of combination of drought and shade stress on turf varieties of tall fescue (*Festuca arundinacea* Schreb.).

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Abiotic stress factors cause extensive losses to plant production worldwide. Individually, each stress conditions have been intensively studied. However, plants in field conditions are often subjected to combination of different stresses as for example drought and heat.
The main goal of our experiment was to discover are there any interactions between two abiotic stresses: drought and shade. Seven tall fescue cultivars were used as well as three other varieties: *Festuca rubra* cv. Salsa, *Poa pratensis* cv. Conni and *Lolium perenne* cv. Aut. Seed was sown on metal pots, 12 pots per one variety. Four combinations of stress conditions were further used: sun and watering, sun and drought, shade and watering and shade and drought. Shade conditions were under natural old tree stand and near building. Watering was withheld after 72 days from sowing (drought condition). Following characters were measured and observed during test: plant performance (1-9 scale, where 9 is full green turf and 1 – no green leaves), above ground matter yield (grams of air-dry matter per pot), number of tillers per area unit, root mass (grams of air-dry matter per pot) for the whole root system and only for roots deeper than 15 cm, root/shoot ratio.
Shade conditions gave completely different results as compared to sun conditions for all traits. First, soil moisture decreased much more slowly in shade than in sun. After 33 days without water soils moisture in sun decreased below permanent wilting point (6.9% of soil moisture). But in shade it took almost 60 days to reach the same level. Therefore, plant performance was also reduced more slowly in shade than in sun. Only less than a half of variation of performance was described by soil moisture in shade (R^2 = 49%) but more than 80% in sun.
The general effect of water deficit on tall fescue varieties ranged from 14.9% (root mass below 15 cm in drought as compared to watering condition) to 85.4% (root/shoot ratio). General effect of shade ranged from 3.6% (root mass below 15 cm) to 37.8% (no. of tillers per unit area). Combined effect of shade and drought gave similar results to shade effect alone: from 0.01% (root mass below 15 cm) to 53.2% (no. of tillers per unit area). Significant interactions of light and water deficit were calculated only of air-dry mass of root system for most of tested tall fescue varieties, but not for Salsa, Aut and Conni. For root system below 15 cm gave significant interaction were calculated for Conni and for tall fescue Asterix, Meandre and Tarmena.
In conclusions, root system of tall fescue seems to be most sensitive to interaction of shade and drought. In case of other traits it seems that plants are able to tolerate both stress factors by developing features conferring reduced demand for light and/or water. Some authors suggest that under limiting light availability (primary limitation), the shortage of another resource such as water should have less impact on plant performance (Canham *et al.*, 1996 after Quero *et al*. 2006).

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Marker assisted selection in forage crops and turf: a review

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Abstract
Although it has been recognized for years that the combination of conventional breeding approaches with molecular tools should assist in making progress in forage and turf cultivar development, in this group of species, cultivars are still being released using conventional breeding approaches (Brummer and Casler 2009). This is in contrast with the increasing number of research reports dealing with DNA-marker assisted characterization of germplasm resources and with QTL mapping for a variety of traits in different species.
In this paper we describe the few attempts so far undertaken to make use of marker assisted selection (MAS) in forage species and offer an overview of the crops and traits on which most efforts were concentrated in the last decades and for which substantial genetic and genomic knowledge is currently available. We further discuss the expected impact of new developments such as association mapping approaches, the availability of relatively cheap high-throughput genotyping technologies and the enormous amount of information available in model organisms.
Achievements in species such as maize or wheat demonstrate the need of international, multidisciplinary collaborations for major advancement. Taking into consideration the level of individual industrial/academic investments currently typical in forage and turf breeding, we will emphasize the relevance of international collaborative efforts and suggest possibilities for resource sharing (i.e., at the level of association mapping populations) for the effective implementation of MAS programs in a typical forage or turf species.

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Allelic diversity for candidate genes and association studies: methods and results

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Abstract

Genetic improvement of crop plants relies on allelic diversity for continued enhancement of existing breeding populations. The increasing ease with which molecular markers can be generated makes it possible for plant geneticists to use these genomic technologies for better exploitation of the available genetic variation. Identifying markers based on conventional bi-parental mapping populations is most likely not the best way to implement a marker assisted selection program, although this approach is useful for introgression of alleles from wild germplasm. Instead, association mapping may be used in a much more practical approach, by measuring both phenotypes and markers directly on the plants in the breeding nursery. Conventional QTL mapping enables one to identify chromosomal regions of 5-20 cM containing genes underlying the trait of interest. However, that still leaves several hundred potential candidate genes. Association mapping enables the exploitation of the wider genetic diversity and incorporate a larger number of recombinations. Synthetic populations used for genetic improvement of self-incompatible crops including many forage and turf species, present a useful tool for incorporating association mapping and genotype building using molecular markers. This is particularly true for traits that have not previously been selected for, since linkage disequilibrium (LD) is less likely to have been built up. We present here methods for candidate gene approaches and association mapping and show some preliminary data from drought and cold temperature experiment to illustrate population structure, LD and associations with candidate genes in synthetic populations not previously selected for this trait. Some recent research on association analysis in forage crops such as perennial ryegrass and red clover are also reviewed.

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Translational genomics in red clover

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Abstract
In an ERANET funded project ('Transleg') we are creating a robust physical map of red clover (*Trifolium pratense*) anchored to the genome sequence of the legume reference species *Medicago truncatula*, and aligned to the clover genetic map. The anchored physical map will facilitate dissection of biological traits, future genetic improvement and marker assisted breeding in this important legume crop. A Phase 1 physical map was constructed using three BAC libraries (*Hin*dIII, 5x coverage; *Eco*RI and *Bam*HI libraries, each at 10x coverage) with FPC software using fingerprints from all three libraries. Of a total 29730 clones, 22987 were placed in 2440 contigs. The largest number (1445) contained 3-9 clones, while 234 contigs contained two clones, and 641 10-24 clones. The rest had over 24 clones. A total of 62599 BAC ends were sequenced (BES) successfully from 36864 clones attempted. A first alignment showed that 792 red clover contigs, using BES and BAC clones, were aligned to the *M. truncatula* reference sequence. Between 150-200 gene specific markers were identified and sequenced in the two red clover parents of the F_1 mapping family. Of those, 132 markers have been put onto a genetic linkage map, and 41 of those have a BAC clone address. A cytogenetics map of red clover has been completed. A pachytene karyogram has been described in which all 7 chromosomes can be identified. An analytical pipeline has been established for using the recently available BES and FPC data, and the repository architecture for storage and retrieval of red clover sequence data has been designed. More genetic markers and their BAC addresses will allow further improvements in the physical map, and in combination with cytogenetics and the analytical pipeline, will provide the robust physical map of red clover needed for establishing a detailed syntenic relationship with *M. truncatula* and the dissection of biological traits.

O28

Application of Diversity Arrays Technology (DArT) for genome profiling of turfgrass

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Abstract

Tall fescue *(Festuca arundinacea* Shreb.) is a turfgrass species a widely used throughout much of the United States because of its adaptation to cooler climates, shade, and ability to maintain lush color year-round in warmer climates with supplemental irrigation. However, germplasm that is available both commercially and in variety trials is surprisingly similar in appearance and performance. To test genetic variation present, we sampled 94 genotypes from the 2006 National Tall Fescue Test in Riverside, CA. DArT analysis revealed only 46 polymorphic markers. While sampling was designed to discover differences in marker presence/absence, as opposed to differences in allelic frequencies, 40 genotypes of forage-type tall fescues tested at the same time differed by over 1,000 DArT markers (Kopecký et al., 2009). Low polymorphism of turf entries indicates a very close relationship regardless of the origin of a cultivar. These data are in agreement with field observations showing very few distinguishable differences in turf quality and leaf texture among the entries, with the exception of Kentucky-31 and Aristotle. These two genotypes rank at the bottom for these traits and show the highest polymorphism of DArT markers. Such low genetic diversity in turf germplasm may have been caused by either a severe genetic bottleneck in the conversion of germplasm from pasture/fodder use to turfgrass, or to extensive sharing of germplasm among breeders. More concerted efforts need to be undertaken to rapidly expand the genetic basis of turf-type tall fescue germplasm if breeding progress is to be sustained.

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O19

Understanding Summer Dormancy and Identifying Associated QTL in Tall Fescue

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Abstract

Summer dormant tall fescues (*Lolium arundinaceum*) survive harsh summers and have better persistence and fall regrowth than summer active plants (Malinowski et al., 2005). Summer dormancy is poorly understood in tall fescue and is often confused with drought tolerance (Volaire and Norton, 2006). Therefore, our objectives are to determine the factors governing summer dormancy in tall fescue and identify the associated QTL. The influence of day length, temperature, vernalization and water stress on summer dormancy in summer dormant, summer active and semi-dormant tall fescue genotypes was studied in growth chambers. Preliminary results indicate long days, coupled with high temperatures, seem to induce summer dormancy as evidenced by reduced tiller number (13 vs 17.1 and 19.6), plant height (17.3 vs 21.3 and 18.3cm), and number of green leaves (10 vs 27.6 and 23.6) in the dormant vs active and semi- dormant genotype. An F_1 mapping population consisting of 210genotypes was developed from a cross between a summer active and a summer dormant parent to identify the QTL governing summer dormancy. The population was planted at two locations with three replications at each location. Significant differences among the genotypes were observed for plant height, yield and regrowth, in summer and fall 2008. Plant yields ranged from 0 to 140 g of dry matter in summer and 0 to 340 g in fall. Genotyping is underway with EST-SSR markers from a reference tall fescue population. Markers that segregate in either parent will be used to genotype the entire population and QTL will be identified using the MapQTL 5.0 software.

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P60

'**Omic' profiling of** *Lolium perenne* **L. genotypes under different N concentrations.**

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Abstract
Improving the nitrogen use efficiency of perennial ryegrass is a target trait for breeders. A panel of seven genotypes has been characterized with respect to their growth response to different concentrations of nitrogen. The metabolic response of these genotypes has been analyzed by GC-TOF-MS to identify metabolites accumulating under different concentrations of nitrogen. In parallel, a macro-array of genes up-regulated under low nitrogen has been created from subtracted libraries. The macro-array has been hybridized with the panel of genotypes in order to profile the expression of these genes with respect to Nitrogen concentration. This has provided an overview of changes in the metabolome and transcriptome in response to nitrogen concentration.

O18

Polyploidization and gene expression in *Medicago sativa*

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Polyploidization is a common event in plant evolution and can influence economically important traits. Modifications in gene expression and/or in DNA sequence are known to occur as a consequence of polyploidization (Chen 2007). The objective of our work is to gain insight into the effects of sexual polyploidization on gene expression in alfalfa, a forage legume with tetrasomic inheritance. We have used two diploid (2x=16) plants of the subspecies *Medicago falcata* and *M. coerulea* that produce 2n eggs and 2n pollen, respectively. From their cross, diploid and tetraploid (4x=32) progenies from bilateral sexual polyploidization (BSP) were obtained. We have used three 2x and three 4x progeny plants to investigate polyploidization-induced changes in leaves of plants in the vegetative phase, by analyzing gene expression level using the *Medicago* Genome Array (Affymetrix), containing cDNA-derived sequences of over 61,000 genes and open reading frames from *M. truncatula* (51,000), *M. sativa* (1,800) and *Sinorhizobium meliloti* (8,300) of *M. trucatula*. Comparisons of gene expression levels were made between the parents and between the midparent and each diploid and tetraploid progeny plant.
Significant expression change as a consequence of polyploidization, and not of just hybridization, was observed for 189 genes. Fewer genes appear to be affected with respect to those affected by allopolyploidization in *Arabidopsis* (Wang et al 2006), but more than those affected by autopolyploidization in *Solanum* (Stupar et al. 2008).
The results of a Gene Ontology analysis of these genes is also presented.

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Characterization of dehydrin variants linked to freezing tolerance in *Medicago sativa* L. at the DNA and post-transcriptional levels.

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Abstract

Breeding alfalfa cultivars with superior tolerance to freezing (TF) could be accelerated by the identification of molecular markers associated to that trait. Dehydrins are highly hydrophilic proteins that are thought to play key adaptive roles with regard to tolerance to freezing-induced cell desiccation (Kosova et al., 2007). A cDNA clone (*msa*CIG; Castonguay et al. 1997) with sequence features typical of dehydrins has been previously isolated from cold-acclimated alfalfa (*M. sativa* cv. Apica). DraI-digested DNA from 45 genotypes of each of the cultivar Apica (ATF0) and populations obtained respectively after two (ATF2) and five (ATF5) cycles of recurrent selection for tolerance to freezing within Apica was hybridized to the *msa*CIG probe. A polymorphic fragment that markedly intensified in response to selection was detected on Southern blots and was subsequently shown to be closely related to freezing tolerance. We hypothesized that the observed polymorphism was the result allelic variation attributable to intragenic variations in the *msa*CIG genomic sequence. Sequence analyses revealed the presence of homologs with insertions/deletions (indel) in the C-terminal region that do not alter the open reading frame. A fragment with a single 60 bp indel was preferentially amplified using pooled DNA from genotypes with the *msa*CIG polymorphism uncovered on Southern blots. Amplification of the same section of the C-terminal region using cDNA templates from cold-acclimated plants confirmed the accumulation of transcripts near the expected molecular size for the polymorphic dehydrin. Western blot hybridization with antibodies raised against dehydrins revealed variations in polypeptide profiles that closely matched the allelic pattern uncovered with DNA amplifications. One band was found to be more abundant in ATF5 than in the initial cultivar. The identification of dehydrin variants closely associated to superior freezing tolerance will pave the way to the development of functional markers.

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O17

The complete chloroplast genome sequence of perennial ryegrass (*Lolium perenne* L.)

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Abstract
To date more than 100 complete chloroplast genome sequences of plants have been published. Ten of these sequences belong to species of the monocotyledon family Poaceae (grasses) which holds many of the world's most important crop species including maize, rice and wheat. However, the complete chloroplast genome sequence of perennial ryegrass (*Lolium perenne* L.) one of the most important forage grass species of the northern hemisphaere was until now not sequenced. Therefore, we sequenced, assembled and annotated the entire chloroplast genome of *Lolium perenne* cv 'Cashel', searched it for single nucleotide polymorphisms (SNPs) and RNA editing sites, and compared it to the chloroplast genome sequences of other Poaceae species.
The chloroplast genome sequence of *Lolium perenne* is 135,282 bp long and encodes for 130 genes. Thirty one RNA editing sites were detected. Six of these editing sites are unique to *Lolium perenne*. Forty SNPs were found within the sequenced sample of 'Cashel', the majority of them within the large single copy region of the genome. The comparison to other Poaceae species showed in general a high degree of conservation regarding genome and gene length, gene content and order. However, 26 genes showed high variation in length with differences of up to 68 codons.
Information on the chloroplast genome of *Lolium perenne* can be used for several applications such as supporting classical breeding schemes to define cytoplasmic breeding pools, for phylogenetic analysis or for genetic engineering. The sequence of the *Lolium perenne* chloroplast genome is available on EMBL/GenBank/DDBJ (Accession number: AM777385).

O27

Exploring the potential for translational genomics approaches in forage legumes: Regions of highly conserved microsynteny between white clover and *Medicago truncatula* revealed by BAC sequencing.

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Abstract

Comparative genomics studies between well characterized and resourced model species and key agricultural species continue to be an important discovery route for the genes underlying important traits in the agricultural species. White clover is closely related to the model legume species *Medicago truncatula*, for which the "genespace" sequence is available. Genetic mapping studies have provided evidence that gene content and order is reasonably well conserved between white clover and *M. truncatula*. (George et al. 2006, Zhang et al. 2007) While these studies have begun to address the macrosyntenic relationships between the genome of white clover and the model species such as *M. truncatula*, less work to date has been performed on the microsyntenic scale. In a previously published study (Febrer et al 2007), we reported the construction of a BAC library of white clover, the end sequencing of approximately 700 clover BACs, and the comparison of these BAC-end sequences (BES) to the *M. truncatula* genome sequence build available at the time. From the BAC-end sequence analysis we found that 14 paired BAC-ends were shown to have the equivalent pairs of *M. truncatula* sequence on the same *M. truncatula* BAC clone or contig sequence within a span of 25 Kb to 200 Kb. We hypothesised that they represented regions of highly conserved microsynteny between the two species. In this follow-up analysis, we have chosen five of these BACs, sequenced them to approximately six-fold coverage, and compared the resulting assembled contigs to their putatively equivalent regions of *M. truncatula*. Sequence-based comparison of the *Medicago* and clover sequences reveals highly conserved gene content and almost complete conservation of gene order and orientation for all five sequences. The results suggest that, at least in some areas of the clover genome, translational genomics approaches for gene discovery using *Medicago* could be highly successful.

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P63

Transcriptional and Metabolic Profiles of *Lolium perenne* L. Genotypes in Response to a PEG Induced Water Stress.

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Water stress is one of the major factors affecting plants growth and productivity. In this study a comprehensive metabolomic approach was carried out to elucidate the mechanisms involved in *Lolium perenne* L. (perennial ryegrass) response to water stress. When leaf and root material from two genotypes, with a contrasting water stress response, were analysed by GC-MS a clear difference was observed in the metabolic profiles of the leaf tissue under water stress. Differences were principally due to a reduction in fatty acids levels in the more susceptible 'Cashel' genotype and an increase in sugars and compatible solutes in the more tolerant 'PI 462336' genotype in response to stress. Sugars significantly increased included; raffinose, trehalose, glucose, fructose and maltose. Raffinose was identified as the metabolite with the largest accumulation under water stress in the more tolerant genotype and may represent a target for engineering superior drought tolerance into perennial ryegrass. The metabolomics approach was combined with a transcriptomics approach in the water stress tolerant genotype 'PI 462336', which has identified genes in perennial ryegrass that are regulated by water stress such as fructan:fructan 6G-fructosyl transferase encoding gene. Additional fructan analysis was performed by HPAEC and confirmed differences in fructan levels between the two varieties. This is the first comprehensive report describing the metabolome of a forage grass under water stress.

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Plant Transcription Factors as Novel Molecular Markers for Legumes

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Legumes include many agriculturally important crops with high protein and oil content, high nutritional value, and the unique ability to fix atmospheric nitrogen through symbiotic relationships with rhizobia. Transcription factors are global regulators of gene expression that play a vital role in many cellular processes and represent excellent targets for developing molecular markers. The goals of this research are to develop a comprehensive resource of plant transcription factors and utilize them to facilitate the transfer of information across multiple legume species. More than 1,000 primers designed from *Medicago truncatula* transcription factor gene sequences (Kakar et al., 2008) were initially evaluated in alfalfa (*M. sativa* L.). Primers producing PCR amplicons in this important forage legume were used to further evaluate amplification in an expanded panel consisting of plant model legumes (*M. truncatula, Lotus japonicus*) and crop legumes (*Glycine max* L., *Pisum sativum* L., *Phaseolus vulgaris* L., *Vigna radiata* L., *V. unguiculata* L., *M. sativa* L., *Trifolium repens* L., *T. pratense* L., and *Lupinus albus* L.) that included parents of existing mapping populations. Amplification, size polymorphism, and sequence variation were evaluated using capillary sequencers. From the total number of primers producing amplicons, 98%, 94%, 47% and 42% of them amplified in *M. truncatula*, *M. sativa* L., *Lotus japonicus* and *Glycine max* L., respectively. In general, the likelihood of successful amplification decreased with increased phylogenetic distance among species. Our study evaluated multiple legume species to identify anchor markers which can be used to facilitate the transfer of information from models to crops. These resources represent valuable tools that can be used to evaluate genetic diversity and for marker-assisted selection in multiple legume breeding programs that include species with limited genetic and genomic resources.

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P65

ISSR markers as a tool for the assessment of genetic polymorphism in *Phleum spp*.

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Abstract

Inter simple sequence repeat **(**ISSR) method is effective and informative method for studying genetic diversity and relationships in timothy species (*Phleum spp*.) This study sought to identify the genetic background of the genotypes used in timothy breeding. A total of 130 individuals from thirteen genotypes of *Phleum pratense*, *Phleum nodosum* and *Phleum phloides* were used as test materials. 137 highly reproducible bands (from 5 ISSR primers) were analyzed. Forty eight bands ranging in size from 0.4 to 2.0 kbp were scored for *Phleum pratense* analysis and forty five of them were polymorphic. The applied primers revealed a total of forty three bands in the range of 0.5 to 2.0 kbp in the *Phleum nodosum* populiation analyzed. Out of forty three bands, forty two found to be polymorphic. Forty five polymorphic bands were detected within *Phleum phloides* populiation. A total number of bands was forty six and they ranged from 0.40 to 2.0 kbp. Differences in the level of DNA polymorphism among the populations were established. Differences in the level of DNA polymorphism among the populations were established. The results demonstrate that ISSR method can be used for assesment of genetic variation in timothy species.

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P66

LD4X: a program to test linkage disequilibrium between loci in autotetraploid species

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Abstract
Autotetraploidy is frequent in forage species (*Medicago sativa*, *Dactylis glomerata*, *Lotus corniculatus*…). Genetic studies may require the test of linkage disequilibrium between loci, in a first step before association mapping (Flint-Garcia et al., 2003). No software currently implements this test in autotetraploid species. We propose a program, LD4X that performs a Fisher exact test between pairs of alleles at two loci (Julier et al., 2009). For each pair of alleles, a contingency 5x5 table with the allelic dosage from 0 to 4 for each allele is built. Then a Fisher exact test is performed to test a departure from equilibrium between these two alleles, giving the probability of significance of the F test. All combinations of alleles from two loci are treated in turn. If two alleles of a pair of loci have a non-random distribution, the markers are considered as linked. LD4X was written using Splus software (Becker et al. 1988), but can be used by the free R software It is adapted to loci scored as codominant, with a real estimation of allelic dosage, but can also be used with loci scored as dominant. The program was tested on a set of microsatellite markers in lucerne (*M. sativa*) synthetic populations. The program is available on request by email to the author. It is also down-loadable on the website www.poitou-charentes.inra.fr/LD4X.

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From a model to a crop species: Constans is involved in aerial morphogenesis of lucerne

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Abstract
Medicago truncatula is a model species for legume crops. It is expected that genomic resources developed in the model species will accelerate genetic analysis in related crop species. We have hypothesised that a gene involved in trait variation in *M. truncatula* could also explain a part of the variation for the same trait in lucerne (*M. sativa*).

Three mapping populations of *M. truncatula*, each of about 200 RILs, were studied over six years in greenhouse. QTLs for flowering date and stem length were identified on chromosome 7 for all populations and years (Julier et al., 2007; Pierre et al., 2008). They explained between 11 and 60% of the variation for flowering date. A meta-analysis of these QTLs and a fine mapping strategy (the analysis of a pseudo-F2 population of 1640 plants) indicated a confidence interval to search for candidate genes. Using sequencing information of *M. truncatula* website (www.medicago.org) and other bioinformatics resources, a list of 573 genes in the QTL confidence interval was established. Among them, six genes (Constans, three copies of FT, PKS and FD) were described in the literature to be involved in flowering date. One of these genes, Constans, was differentially expressed in two parental lines with contrasting flowering date.

This gene was used as a candidate for association genetics in lucerne. Primers were designed to amplify two regions of about 500 bp each. Direct sequencing of PCR products was performed in a population of 400 lucerne genotypes. The genotypes were evaluated for flowering date and plant height in two locations during four years. Only eight SNPs with a frequency higher than 0.1 were identified (1 SNP every 125 pb), which was clearly less than in a neutral gene (1 SNP every 30 pb). SNP frequency was lower in Constans than in a neutral gene (1 SNP over 125 bp *vs*. 1 SNP over 30 bp, respectively). Eight SNPs with a frequency higher than 10% were identified. Two of them were significantly associated to flowering date and plant length recorded in some conditions. Constans is thus also involved in plant morphogenesis in lucerne.

The use of a model species to identify genes involved in trait architecture is valuable. These genes are good candidates to explain variation in crop species.

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P68

QTL for water use efficiency in alfalfa

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Abstract

Alfalfa is the most important forage crop cultivated in semi-arid areas, both in rainfed and irrigated conditions. In the two situations, water available for crops is a limited resource and improvement of water use efficiency (WUE) a general goal for plant breeding. WUE is the efficiency of a plant or a crop to transform transpired water into biomass and yield. The objective of the study was to detect QTL for WUE in a mapping population of alfalfa.

A F1 mapping population of 224 individuals was obtained by crossing two plants originating from South of France (Magali) and from Tunisian oasis (Gabes). SSR markers were tested for amplification and polymorphism among the parents. Eighty five markers were used, generating 280 alleles. The map, calculated with TetraploidMap software, comprised 8 linkage groups for each parent. Each plant was transplanted in a soil column (height 2 m, diameter 8 cm). After full establishment (1 year, 4 cuts), WUE was measured along six regrowth cycles under well-watered conditions. Aerial dry matter (DMA, g) was measured at each cut. Each column was conducted as a lysimeter: they were weighted at each cut, water supply and drainage between two cuts were also weighted. Evapotranspiration (ET, g) was calculated from the water balance for each plant and each growth cycle. WUE for each plant was calculated $(DMA/ET)*10^3$. Average value of WUE over the six cycles was calculated. QTL detection was carried out by analysis of variance. In a first step, a simple ANOVA with each allele was made. The significant alleles were then submitted to a multiple ANOVA, using the option SS2 of proc GLM of SAS.

The two parents significantly differed for WUE under irrigated conditions as expected (Gabes had a higher WUE than Magali). The F1 population showed a quantitative variation for this trait. In simple ANOVA, nine markers/alleles had a significant effect on WUE variation. In multiple ANOVA, six markers/alleles had a significant effect and explained 31.0% of the variation. They were located on chromosomes 2, 3 7 and 8. This QTL detection will be completed by the analysis of other traits, as WUE in dry conditions and drought tolerance in field conditions. The QTLs will be useful to better understand adaptation to water stress conditions in lucerne and to breed improved varieties.

Acknowledgements

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QTL Mapping of Aluminum Tolerance in Tetraploid Alfalfa

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Abstract

Aluminum (Al) toxicity in acid soils is one of the factors limiting crop production. Alfalfa (*Medicago sativa* L.) is one of the most important forage legumes worldwide and is susceptible to Al toxicity. Al-tolerance in alfalfa was identified in a diploid *Medicago sativa* subs*. coerulea* accession (Sledge et al., 2002) and has been successfully integrated at the tetraploid level. The goals of this study are to identify and confirm quantitative trait loci (QTL) for Al-tolerance in tetraploid alfalfa (Altet-4). Two populations of at least 190 individuals each were developed from crosses between Altet-4 (Al-tolerant) and the Al-susceptible genotypes 95-608 derived from CUF-101, and NECS141, a semi-dormant breeding line developed in Iowa. The parental lines and the progeny from the mapping populations were screened using a callus bioassay and whole plant assay. Genetic linkage maps constructed using EST-SSR markers (Sledge et al., 2005) were developed and used to identify QTL associated with Al tolerance. Comparison of screening methods and colinearity of QTL between diploid (Narasimhamoorthy et al., 2007) and tetraploid alfalfa will be discussed. In addition to performance of diploid Al-tolerance, the type of 2n gamete (First division restitution and Second division restitution) will be defined to provide a better understanding of Al-tolerance in this tetraploid alfalfa. The long-term goal of this research is to use molecular markers linked to the Al-tolerance trait and accelerate the development of Al tolerant alfalfa cultivars.

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Oral

DArTFest - a platform for high-throughput genome profiling within the *Festuca – Lolium* complex

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Grasses are among the most important and widely cultivated plants on the Earth. They are grown for grassland and silage production on arable land. Among cultivated grasses, species within the *Festuca-Lolium* complex predominate, especially in temperate regions. With the aim to facilitate high-throughput genome profiling and genetic mapping within the complex, we have developed a Diversity Arrays Technology (DArT) array for five important species: *F. pratensis*, *F. arundinacea*, *F. glaucescens*, *L. perenne* and *L. multiflorum*. The DArTFest array contains 7680 probes derived from methyl-filtered genomic representations. In a first marker discovery experiment, performed on 40 genotypes from each species (with the exception of *F. glaucescens* for which only 7 genotypes were used) we identified 3884 polymorphic markers. The highest number of markers was identified in *F. pratensis* (1619 to 1821 markers per accession) and *L. multiflorum* (1507 to 1852 markers per accession). Lower numbers of markers were discovered in *F. arundinacea* (1000 to 1351 markers per accession), *F. glaucescens* (1059 to 1101 markers per accession) and *L. perenne* (821 to 1127 markers per accession). In order to assign DArT markers to individual chromosomes and chromosome bins of *F. pratensis*, we have developed a series of single chromosome substitution and recombinant lines of *F. pratensis* in tetraploid *L. multiflorum*. The use of recombinant lines with different sizes of introgressed *Festuca* chromosome segments enabled mapping DArT markers to particular chromosome regions. The resources developed in this project will facilitate development of genetic maps in *Festuca* and *Lolium*, and to follow genomic constitution in *Festuca* × *Lolium* hybrids during their breeding. Moreover, it should enable simultaneous marker-assisted selection for multiple traits or specific genome regions. This work was supported by the Ministry of Agriculture of the Czech Republic (grant award NAZV QH71267) and the Czech Science Foundation (grant award 521/07/P479).

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Differences in leaf proteome response to cold acclimation in two *Festuca pratensis* plants distinct in the level of frost tolerance

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Abstract

Festuca pratensis Huds. as the most frost tolerant species within the *Lolium-Festuca* complex can serve as a model plant for the research on the mechanisms of cold acclimation (CA) and frost tolerance in forage grasses. Two *F. pratensis* individuals with contrasting levels of frost tolerance after 21 days of CA, *Fp13* (LT_{50} = –15.9°C) and *Fp37* (LT_{50} = –21.4°C) as a low frost tolerant (LFT) plant and a high frost tolerant (HFT) plant, respectively, were selected for comparative proteomic research. The work was focused on the analysis of protein expression profiles before CA and after 2, 8 hours, and 2, 4, 6, 7, 14 and 21 days of CA using a high-throughput two-dimensional electrophoresis and on the identification of proteins which were differentially accumulated between the selected plants by the application of tandem mass spectrometry. The analyses of approximately 800 protein profiles revealed a total of 41 (5.1%) proteins that showed at least 1.5-fold differences in abundance at least at one time point of CA between HFT and LFT individuals. In the case of 19 proteins the differences were observed only at one time point of CA, mostly on the 2nd day of CA. Nine proteins showed differences in abundance at two different time points of CA, five proteins at three time points, one protein at four, four proteins at five and three proteins at six different time points of CA. Among them, the time point at which most often the differences were observed was the 2nd day of CA. The identified proteins were mainly involved in photosynthesis, energy production and translation in chloroplasts.

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Vegetative plant height QTLs in elite perennial ryegrass material.

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Abstract
Perennial ryegrass (*Lolium perenne*) is the first sown forage grass in temperate regions. It is choosen for its high appetibility, its good digestibilty and its adaptation to pasture (Wilkins and Humphreys, 2003). Leaf length in perennial ryegrass meadows is a limiting factor for feeding grazing cows (McGilloway *et al.*, 1999). In spite of the phenotypic selection efficiency to improve this trait, synthetic varieties hamper fixing favourable alleles. Our aim was to detect QTLs of vegetative plant height which is highly correlated to leaf length in order to start molecular assisted selection. Two hundred plants from a cross between two elite plants, Nemo B and Nemo F, were used to build a genetic map for each parent with 65 SSRs and 47 AFLP markers. The maps consisted of seven linkage groups for both parents, with a length of 675 cM and 663 cM for Nemo B and F, respectively. Plant height was measured in a nursery on spaced plants during spring 2008. Using the Composite Interval Mapping method, we detected one QTL of plant height with a strong effect (from 13 to 25% of phenotypic variance) in the parent Nemo B on linkage group 2. This QTL co-localized with a QTL of earliness in vegetative growth after winter explaining 21% of variance. Moreover, two QTLs of plant growth rate were found on linkage groups 4 (Nemo F) and 7 (Nemo B), each of them explaining 11% of variance.

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P72

Identification of Molecular Markers Associated with Stem Rust Resistance in Tall Fescue

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Abstract

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh. (=*Festuca arundinacea* subsp. *arundinacea*)] seed production is affected by stem rust caused by *Puccinia graminis* Pers.:Pers. subsp. *graminicola* Z. Urban (Barker et al., 2003). Identification of molecular markers associated with genetic host resistance would accelerate gains obtained using recurrent selection techniques. The objective of this study was to identify QTL and molecular markers associated with stem rust resistance in tall fescue. The Noble Foundation mapping population from a cross between HD28-56 and R43-64 consisting of 124 genotypes was phenotyped under field (Ardmore, Oklahoma) and greenhouse (USDA–ARS, Corvallis, Oregon) conditions. Field stem rust was from natural inoculum while greenhouse plants were inoculated with stem rust spores collected from Oregon fields. Single marker analysis showed that the marker CNL081 segregated between the tolerant and susceptible genotypes of the mapping population in a one-to-one ratio (R^2 = 0.69, $P \leq$ 8.57e-24). Based on the female parental map, composite interval mapping consistently identified three QTL across test times on LG10, LG18 and LG19 for pustule size. The QTL on LG19 also controlled infection type and latent period and reduced pustule size and infection type by 24.6 and 7.8%, respectively, and increased latent period by 6.2%. Another QTL on LG1 was consistently associated with latent period and infection type. Based on the male parental map, two QTL were identified on LG13 and 20 that reduced pustule size by 27.4 and 20.5%, respectively. Markers associated with these QTL are under verification in different populations. Verified QTL and markers will be used for marker-assisted selection (MAS) to reduce the need for fungicides to control stem rust in tall fescue seed production (Pscheidt, 1996).

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P73

Mapping Drought Tolerance in Tall Fescue Populations

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Abstract

Tall fescue [*Lolium arundinaceum* (Schreb.) Darbysh.] growth and persistence are adversely affected by the hot-dry summers in the Southern Great Plains (Hopkins, 2005). Both forage yield and drought tolerance are difficult to select for because of large genotype-by-environment interactions. The objective of this project was to construct, phenotype in multiple locations, and genotype drought mapping populations, and carry out quantitative trait analysis to identify markers for marker-assisted selection. A mapping population was constructed based on a set of genotypes (B400 × W279) contrasting for drought stress tolerance. The population was planted in two Ardmore, Oklahoma field locations, in a greenhouse and at Logan, Utah. To date, data have been collected on relative water content (RWC), cell sap osmotic potential (OP), canopy temperature, senescence, and aboveground shoot production in Ardmore and Utah locations. Significant variation (P<0.0001) for genotypes was found for all traits tested. The population varied substantially for RWC in Ardmore (41.5 to 65.1%, mean 55.1%) and in Logan (32.6 to 86.2%, mean 53.9%). Increased RWC under water deficit is associated with increased drought tolerance (Elmi and West, 1995). The mapping populations are undergoing genotyping with framework microsatellite and STS markers obtained from a tall fescue reference map. In addition, candidate drought-associated markers have been identified and are being incorporated in the genotyping. Single marker analysis indicated that NFFS155, NFFG423 and NFFG106 were associated with shoot dry weight under drought stress both in Utah and Ardmore with R-squares of 0.32, 0.22 and 0.22, respectively.

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P74

Genotyping unknown genomic terrain in complex plant genomes

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Abstract

Non-sequencing based high throughput genotyping (HTG) are today commonly used for genotyping in human and model organisms. Several of these platforms allows for custom designed genotyping chips well suited for genotyping of non-model organisms. The HTG platforms are based on diverse arrays of molecular assays; however there is no straightforward way of ensuring that the results reflect the true genotype at the locus under investigation. One of the biggest potential sources of genotyping errors derives from duplicated blocks of homologous DNA. In organisms with sequenced genomes genomic regions associated with recent gene duplications are identified and discarded in order to avoid paralog- and multisite variation (PSV and MSV). PSV and MSV arise from SNPs between non-homologous loci. Plant genome evolution is highly plastic and DNA duplication events are more common in plants than in animals. As a result, plants have more and larger gene families than other eukaryotes. Genotyping of non- sequenced plant genomes therefore inflates the problem associated with PSV and MSV compared to genotyping of animal genomes. Here, we address the problem of MSV and PSV when HTG is performed in unknown genomic terrains of non-model plant genomes, typically our forage grass species. Through a case study in *Lolium perenne* we show how unknown paralogs can generate DNA variation patterns which reflect a complex mix of paralogous loci. We discuss precautions that can be taken to avoid MSV and PSV analysis and comment on the future prospects of genotyping in complex plant genomes.

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P75

A framework genetic linkage map of *Lolium* based on EST derived SSR markers

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Abstract
Ryegrass (*Lolium* spp.) is the most widely used temperate forage grass species. In recent years, molecular markers for the use in plant breeding programs have received considerable attention. Furthermore, a number of mapping populations of *Lolium* spp. assessing various traits have been established. However, the low number of common markers between mapping populations limits comparative mapping and the ability to infer direct coincidence of chromosome regions and QTL.
Recently, 25,744 perennial ryegrass expressed sequence tags (ESTs) representing 8.53 megabases of nucleotide information have been used to identify 955 (3.7%) ESTs containing non redundant SSR motives (Asp et al. 2007). Out of 744 ESTs which were used for primer design, 464 successfully amplified EST-SSRs. These have previously been reported along with the EST sequence and its gene annotation (Studer et al. 2008).
Here, we report on the use of these EST-SSR markers within a consortium of several grass breeding and research institutions (see affiliations). Specifically, the markers are (1) characterised for their polymorphic information content based on six to eight individuals representing eight different ryegrass mapping populations, (2) used for the establishment of a high density public reference linkage map as a dedicated tool for comparative mapping and finally, (3) used in a map based comparison of previously identified QTL for crown rust resistance among the most important forage and turf ryegrass mapping populations.

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Blind mapping of genic DNA sequence polymorphisms in *Lolium perenne* L. based on high resolution melting curve analysis

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Abstract
High resolution melting curve analysis (HRM) measures dissociation of double stranded DNA from a PCR product amplified in the presence of a saturating fluorescence dye and unlabelled primers. HRM analysis is an easy, fast and closed-tube procedure in one step. Recently, HRM proved to be successful to genotype DNA sequence polymorphisms such as SSRs (Mader et al., 2008) and SNPs (Lehmensiek et al., 2008) based on the shape of the melting curves.
We used HRM for simultaneous scanning and genotyping of genic DNA sequence polymorphisms in the *Lolium perenne* F2 mapping population VrnA. Melting curve shapes successfully discriminated genotypes in absence of allelic sequence information. Allelic segregation patterns of different complexity levels (up to four segregating alleles) were detected over three generations in VrnA for SNP and SSR markers of recently published sequences (Studer et al., 2008) as well as two novel genes involved in vernalization and self incompatibility. Assigned genotypes were in accordance with data previously generated by capillary electrophoresis and the Illumina GoldenGate™ technology. Hence, HRM has the potential to substitute other techniques for SSR, InDel and SNP analysis and is particularly useful for genetic linkage mapping.
Here we introduce the concept of blind mapping based on HRM and report on a powerful, fast and cheap method to map any gene of interest without knowledge of allelic sequences in the key grassland species *Lolium perenne* L.

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S-locus genotyping and preliminary mapping in White Clover (*Trifolium repens* L.)

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Abstract
White clover (*Trifolium repens* L.) is a member of the Leguminosae flowering plant family and is used as a high quality forage crop capable of fixing atmospheric nitrogen. It is an amphidiploid (2n = 4x = 32) species constructed of two ancestral genomes, *T. palescens* and *T. occidentale*. *T. repens* possesses a self-incompatibility mechanism which prevents self-seed setting. The self-incompatibility reaction is contolled by a single poly-allelic locus expressed gametophytically in the male, where common allele specificity in pollen and stylar expressed genes results in late-acting, pre-fertilisation arrest of pollen-tube growth (Atwood, 1940).
Based on the assumption that both parents were heterozygous for unique S-alleles, ninety four individuals of a bi-parental mapping family were classified into four intra-incompatible groupings from partial diallel crosses and pod-filling assessments six to nine days post-pollination of emasculated florets.
Initial analysis of the two parental maps based on AFLP and SSR markers has identified marker associations with the S-locus attributing it to linkage group E2 as classified by Barrett et al (2004). To date, 12, 8, 6 and 16 plants have been assigned the S genotypes S_{13}, S_{14}, S_{23} and S_{24} respectively. Further work will place more markers on this linkage group, and polymorphisms of candidate genes/proteins will be compared to the S-locus genotypes of the characterised plants.

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P78

Fine mapping of quantitative trait loci for biomass yield in perennial ryegrass

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Abstract
Perennial ryegrass (*Lolium perenne* L.) belongs to the Poaceae family and is a main forage grass in temperate zones. It is principally cultivated for permanent pastures to provide a high digestibility forage but is also an important turf and amenity grass species. Improving biomass yield is still one of the most important breeding objectives. Current breeding programmes are using conventional breeding schemes to improve biomass yield and do not rely yet on the application of biotechnology based methods like marker assisted selection (MAS) because of the complex nature of this trait. Biomass yield is controlled by multiple genes and several genomic regions are involved in biomass yield. The trait is as well influenced by environmental interactions. Therefore the study of this trait relies on Quantitative Trait Loci (QTL) mapping.
In a precursor study, a genetic map was prepared from 360 genotypes of an F2 population segregating for biomass yield. Single Sequence Repeat (SSR) and Amplified Fragment Length Polymorphism (AFLP) markers were mapped on the seven linkage groups (LGs) (Anhalt et al., 2008). Three major dry weight and fresh weight biomass QTL were detected consistently under different environments and replications. They were located on LGs 2, 3 and 7 (Anhalt et al., 2007). Current work focuses on the fine mapping of these QTL positions by using additional ryegrass SSR markers and sequence tagged site (STS) markers designed from rice. This approach is possible since a high degree of syntenic relationship was observed between the two species (Sim et al., 2005). Synteny approaches have shown to be very successful to pinpoint genes underlying QTL for agronomical important traits.

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O26

Dissecting *Festulolium* chromosome 3 to locate rooting and drought resistance traits.

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Abstract

Festuca species have better rooting and water extraction characteristics than *Lolium* and this is believed to contribute to their greater drought resistance (Durand et al., 2007). The deeper and stronger roots of *Festuca* species may also increase soil porosity and hydraulic conductivity and, by reducing runoff, lessen the impact of diffuse pollution on water catchments. Inter-generic hybridization between *Festuca* and *Lolium* followed by backcrossing can be used to transfer new trait variation into *Lolium* and, at the same time, dissect the genetic regulation of the traits (Humphreys et al., 2006). Drought-resistance traits have been transferred from two *Festuca* species (*F. arundinacea*, and *F. glaucescens*) onto *Lolium* chromosome 3 of drought-sensitive *L. multiflorum*. *Festuca*/*Lolium* chromosome 3 has conserved DNA sequences in common with rice chromosome 1 along its entire length (King et al., 2002). As rice chromosome 1 is implicated strongly in drought resistance and carries QTL for a range of rooting and soil penetration traits this aids the design of markers for following introgression of rooting and drought traits in *Festulolium*.

Three drought resistant introgression lines and the recombination series derived from the relevant *Festulolium* substitution line together provide good coverage of chromosome 3. These plants have been grown in 1m deep pipes of compost and drought imposed by withholding water. Plant water status was monitored, and above and below ground growth were measured. A range of rooting traits was scored non-destructively. These included maximum root depth, and both root scores and the proportion of new roots in each 10cm horizon. Further rooting traits were derived from the data; in particular root system size and profile. Early results confirm the presence of genes controlling drought resistance and rooting traits on chromosome 3. There were significant effects on the growth of new roots during and just after a period of drought.

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P79

Testing a QTL index for marker selection of water-soluble carbohydrate content in perennial ryegrass.

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Abstract

Perennial ryegrass shows considerable genetic variation for water-soluble-carbohydrate (WSC) content (Humphreys, 1989; Turner et al., 2002). This grass is currently widely used for livestock production in temperate agriculture as the WSC can provide the energy needed for efficient utilisation of forage protein during rumen fermentation. In the future grasses with high carbohydrate content may also be important for use in the production of bioenergy. Variation in the WSC content of ryegrass has been further characterised by quantitative trait locus (QTL) mapping. Several regions of the genome with basic control over carbohydrate metabolism were identified. To benefit future breeding programmes this increased understanding of the genetic control of sugar metabolism must be applied to develop markers that can be used for selection. Candidate genes which might explain these QTL have not been identified (Turner et al., 2006). Therefore the most appropriate way to proceed at present is with random markers from within the genomic regions of interest.

The main QTL regions identified have been validated by individual test crosses. However, in general, only limited extents of the large variation available within the source population was exploited by the individual selections. This highlights some of the challenges ahead for the use of marker selection in breeding programmes handling complex traits in outbreeding crops.

More recently further experimental crosses have been carried out to test the potential of a QTL marker index to exploit the available variation. Parental groups were chosen on the basis of a index weighted for the size of the different QTL. The progeny plants have been maintained in a glasshouse and samples taken for WSC analysis over a three-year period. Carbohydrate content has been measured in both leaves and tiller bases in early spring and in autumn.

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P80

Functional analysis of genes involved in cell wall biosynthesis of the model species *Brachypodium distachyon* to improve saccharification

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The grass family is up to now the most important plant family as resource for the production of first generation Bio-Ethanol, which is solely based on the fermentation of starch and sucrose. These sugar compounds are mostly obtained from food crops like corn (US), wheat (Europe) or Sugarcane (Brazil). If Europe wants to meet the new energy directive, it will be necessary to make the transition from first to second generation bio-ethanol for which recalcitrant lignocellulosic biomass is used as feedstock. The energy resource of lignocellulosic biomass is largely entrapped in the plant cell wall which, is built up from cellulose, hemicellulose and lignin and can make up to 70 % of total biomass. To be able to produce ethanol from these rigid cell walls, the cellulose and hemicellulose need to be degraded into C5 (xylose, …) and C6 (glucose, …) sugars. For the moment, this degradation constitutes a bottleneck in the process. Especially lignin is a limiting factor as it reduces the accessibility of cellulose and hemicellulose to cellulases and binds irreversibly with cellulases. An interesting approach to improve energy crops for the production of bio-ethanol is therefore to modify the lignin content or structure if their cell walls.
In this study *Brachypodium distachyon* is used as a model to study the effect of up- or down regulation of genes with a key-role in the monolignol biosynthesis pathway on the saccharification efficiency. The general strategy and preliminary results of this study will be discussed.

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Identification of genes involved in bacterial wilt resistance in *Lolium multiflorum*

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Abstract

Xanthomonas translucens pv. *graminis* (*Xtg*) causes bacterial wilt in many forage grasses including Italian ryegrass (*Lolium multiflorum*), seriously reducing yield and quality. Breeding for resistance is currently the only practicable means of disease control. Molecular markers closely linked to resistance genes and QTL can complement and enhance phenotypic selection. We used comparative gene expression analysis of infected and non-infected *L. multiflorum* genotypes differing in susceptibility to bacterial wilt to identify genes involved in the control of *Xanthomonas* resistance. Fluorescently labelled cDNA prepared from plant leaves collected at four different time points after infection was hybridized to a cDNA microrarray containing 10'000 unigenes from *L. perenne*. Comparisons and statistical analyses of the gene expression profiles of the resistant genotype revealed 0, 20, 52 and 124 up-regulated and down-regulated genes 8h, 48h 192h and 288h after infection compared to non-infected controls and considering a p-value threshold of 0.01. Up-regulated genes included genes with high sequence similarity to germin-like proteins (GLPs), a gene family known to be involved in the regulation of basal host resistance. Validation of selected differentially expressed genes with real-time PCR is in progress. In contrast to the small number of genes differentially expressed upon infection in the resistant genotype, comparison gene expression profiles of the resistant and the susceptible genotype revealed more than 400 differentially expressed genes; this large number will render the assignment of genotypic differences responsible for host resistance difficult. Nevertheless, the genes differentially expressed upon infection will serve as the basis for the identification of key genes involved in bacterial wilt resistance and to develop molecular markers for marker assisted breeding.

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Mapping and marker assisted selection of genomic regions associated with nitrogen use efficiency and other forage productivity traits in *Lolium perenne* cultivars.

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Breeding forage grasses capable of using nitrogen (N) fertiliser inputs more efficiently offers a clean technology route to increased sustainability of livestock production, via lowering recommended fertilizer rates, reducing the agricultural footprint with respect to pollution and reducing the wider consumption of non-renewable resources. To identify and incorporate new genes associated with nitrogen use efficiency (NUE) in perennial ryegrass (*Lolium perenne*) cultivars, we are making use of a research programme in which genetic mapping and marker assisted selection of traits are practiced simultaneously. A mapping population in the genetic background of the current elite cultivar AberMagic has been developed which segregates for N uptake and N utilisation efficiencies as well as a number of forage quality and productivity traits. By phenotyping this population under highly controlled optimal and limiting regimes of N supply in flowing solution culture, a number of genomic regions mapping to linkage groups 1, 2, 5 and 7 have been identified affecting nitrogen relations. Genomic regions segregating for a number of other desirable agronomic traits such as flowering time, growth and re-growth after cutting, and forage quality traits such as water soluble carbohydrate content, dry matter digestibility, and concentration of polyunsaturated fatty acid (PUFA) in the forage have also been identified. A major advantage of this population is that it has been developed in the genetic background of an elite cultivar allowing identification of new alleles of traits that are not present in this cultivar/gene pool. Using such a population provide a way of fast-tracking introgression of new alleles from the donor ryegrass genotype into AberMagic and other cultivars originating from a similar gene pool. Progress so far in the mapping and marker assisted breeding of new alleles identified so far will be discussed in the context of developing new varieties.

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Association mapping of forage quality traits in perennial ryegrass

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Abstract

The association between a set of validated 144 SNPs from 21 genes involved in herbage quality variation (lignin biosynthesis and oligosaccharide metabolism) and seven forage quality traits were studied using an association mapping panel (AMP) of 480 perennial ryegrass individuals. The AMP consisted of 384 perennial ryegrass individuals, selected from globally diverse germplasm (192 genotypes) and from ecotypic materials (64 genotypes from each of the Kangaroo Valley, Victorian and Zürich Uplands populations) was assembled, and was augmented by 48 genotypes from each of the cultivars Tolosa and Bronsyn. Population structure was investigated using 60 locus-specific simple sequence repeat (SSR) loci distributed across the genome (both genomic DNA-derived SSRs and EST-SSRs). Both PCoA and clustering analysis showed four distinct clusters. The Tolosa, Bronsyn and Zürich Uplands populations formed separate clusters and all the other populations are in a single cluster. Analysis of molecular variance (AMOVA) showed 91% variation within all populations as compared to 9% between populations. When PCoA and clustering analysis were applied to the megapopulation consisted of genotypes from diverse population, Victorian and Kangaroo Valley, no clustering tendency is obvious. AMOVA indicated that 99% of the variation was within populations and only 1% between populations. Therefore, the megapopulation was treated as a 'meta-population' for association analysis. Definition of the 'metapopulation' was also revisited through principal coordinate analysis (PCoA), which evaluated up to 70% of genetic variation in the first three axes; through non-parametric clustering, which revealed the stability of the metapopulation through data 'boot-strapping' at greater than 90% confidence; and parametric clustering, which allowed finer resolution of population sub-structure. The Zürich Uplands sub-population was sub-divided into up to 6 groups, which may relate to ecogeographical differentiation, and hence represent further potential sources of rare and valuable alleles. Association analysis of the meta-population was based on traits measured at the reproductive stage, and several models implemented with individual SNP loci, as well as haplotype analysis for the full-length genes *Lp*FT1, *Lp*FT3 (*Lp*1-SST), *Lp*CCR1 and *Lp*CAD2. Correlations for lignin-associated traits were observed with a number of genes, especially the *Lp*CCoAOMT1, *Lp*OMT1/3, *Lp*CCRb and *Lp*CCR1 and *Lp*CAD2 genes, all of which are located on LG7 of perennial ryegrass, as well as the *Lp*CELL cellulose gene, which maps to LG4. Herbage quality QTLs have been previously reported to co-locate with the *Lp*OMT1/3, *Lp*CCR1 and *Lp*CAD2 genes. Several oligosaccharide metabolism genes also show significant associations with digestibility traits, presumably due to carbon partitioning effects. The *Lp*SPSf (sucrose phosphate synthase) and *Lp*FT1 (fructosyltransferase) genes also showed associations with WSC and starch content, respectively. All of the observations were consistent with prior knowledge of herbage biology. Haplotype association analysis was also attempted, but produced less consistent results.

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Identification of QTL associated with morphological and agronomic traits in white clover (*Trifolium repens* L.)

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Abstract

White clover (*Trifolium repens* L.) is an important cool-season perennial forage legume species used in pastures to improve forage quality. The identification of molecular markers linked to morphological and agronomic traits could facilitate the development of superior white clover cultivars. The objectives of this study were to map quantitative trait loci (QTL) associated with morphological and agronomic traits using a F_1 population from a double pseudo-testcross between two highly heterozygous genotypes GA02-56 derived from Durana (Bouton et al., 2005) and GA02-15 derived from SRVR (Gibson et al., 1989). Phenotypic data was collected from multiple field locations and years for morphological traits (leaf length and width, petiole length, stolon diameter and inter-node length), and for growth traits (plant spreading, plant height, and stolon number). Analysis of variance indicated there were significant effects from location, replicates within location, genotype, and genotype x location for all traits evaluated. Correlation coefficients showed that growth traits in the field were highly correlated with each other. Broad sense heritability estimates for all traits evaluated were less than 25%. The population was genotyped using simple sequence repeat (SSR) markers (Zhang et al., 2008) and multiple QTL model (MQM) analysis was used to identify 37 QTLs on eight linkage groups associated with five morphological traits and four plant growth traits. The consistent location of QTL for the same traits or highly correlated traits across different locations and years indicates the potential value of utilizing marker-assisted breeding for white clover improvement.

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Festulolium hybrids: results, limits and prospects

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Abstract
The *Lolium-Festuca* complex represents a vast resource of variant alleles for novel agronomic and adaptive gene combinations for exploitation as grasslands for sustainable agriculture, environmental security, or alternative amenity and leisure purposes. This is achieved through the assembly in interspecific hybrids of desirable genes within a common germplasm either by stabilising their genome integrity and promoting disomic inheritance through amphiploidy, or by encouraging promiscuous genome recombination and monitoring the movement of key alleles into subsequent generations by marker-assisted selection. The incorporation of high-throughput genomic and phenomic technologies in crop improvement programmes makes possible the full exploitation of the genomes of all interfertile *Lolium-Festuca* hybrids.
This was encouraged by 2004 EU legislature which now characterises *Festulolium* to include varieties derived following hybridisation between any *Lolium* and *Festuca* species on the proviso that this was achieved by conventional breeding technologies. To date, 28 *Festulolium* varieties have been registered at least once in National, EU or OECD lists of which 16 were allotetraploid *L. multiflorum* × *F. pratensis* or *L. perenne* × *F. pratensis* hybrids, and the remainder introgression lines, 6 primarly *Lolium*, and 6 *F. arundinacea*, dependant on the recurrent parent species used in the back-cross breeding programme (Kopecky et al. 2006).
Introgression mapping for complex traits such as drought resistance or winter hardiness has been made possible in hybrids between *Lolium* and *Festuca* species by exploiting their capability for interspecific genome recombination in order to "dissect" the key components, and then to monitor their transfer over generations using genomic *in situ* hybridization (GISH), (Thomas et al., 2003). Furthermore integrated approaches to combine genetic and physical mapping with comparative mapping of syntenic loci from sequenced genomes of model crop species is allowing knowledge transfer of genes controlling major traits for the benefit of all monocot crops, and also improved knowledge of genome evolution, structure, relationships, and recombination both within the *Lolium-Festuca* complex and other *Poaceae* crop species (Yamada et al., 2005).

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Creation of heterotic groups and hybrid varieties

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Abstract
Capturing heterosis for dry matter yield in forage crops needs to consider both the final product of the breeding process (the type of cultivar) and the breeding method used to produce the cultivar. The discussion will be focused on tetraploids, specifically alfalfa, and on the semi-hybrid variety model allowing partial expression of heterosis with the technology currently in use and no drawbacks to seed production.
The effectiveness of the process of semi-hybrid development is dependent on the breeder being able (i) to concentrate in parental populations favourable genes and linkats by means of recurrent selection and/or selfing and selection and (ii) to capture in their crossing significant and positive combining abilities (i.e., specific combining ability) upon which heterosis of quantitative traits is based.
Genetic diversity between the parental populations crossed is a prerequisite for heterosis expression; however, the divergent populations must also be "good combiners" in order to observe high hybrid vigour for dry matter production. The search for heterotic groups is a key point to improve the process of semi-hybrids construction. Among-subspecies diversity (i.e., *M. sativa* subsp. *sativa* and *falcata*) and selfing within subspecies are tools to enlarge genetic diversity and to test the value of gene and linkat interactions in crosses. The use of reciprocal recurrent selection to develop adapted, elite populations that are also effective combiners should also be considered.
The amount of hybrid gain with respect to the better producing parent population and to the corresponding synthetic variety (i.e., the synthetic made by the same parents used in semi-hybrid construction) needs to be considered. A synthetic derived from hand crossing the original parents may be an even superior method to realize heterosis in the field. Regardless, the key ingredient is to not intermingle the heterotic groups once developed. The respective role of general and specific combining abilities in semi-hybrids, the effects on interplant variation for agronomic traits), the heterosis expression at individual plant level, and the expression of heterosis at the molecular level assayed by microarray technique are all interrelated and will be considered.

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Enhancing the adaptation to Italian environments of Egyptian lucerne germplasm for exploitation as a component of free-hybrids

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Abstract
Lucerne may show large heterosis for forage yield mainly because of non-additive, complementary gene interactions between different alleles. Free-hybrids between genetically-distant, well-complementing populations previously subjected to separate selection have been proposed for enhancing heterosis (Brummer, 1999). The homogeneity requirement for variety registration in EU discourages the use of *M. sativa* × *M. falcata* free-hybrids, suggesting to locate heterotic populations within geographically-distant germplasm pools of *M. sativa*. The Egyptian germplasm is a candidate component of free-hybrids in Italy, because of its history of cultivation in relatively isolated environments such as the desert oases. However, exotic candidate components of free-hybrids need undergo selection for local adaptation, which, for Egyptian germplasm, mainly concerns improved winter hardiness. After a preliminary selection stage encompassing one winter in an open cold greenhouse (Carelli et al., 2008), 74 genotypes belonging to 17 Egyptian landraces and two Egyptian varieties and 74 genotypes selected from a locally well-adapted Italian landrace were evaluated in northern Italy as replicated clonal material for dry-matter yield, disease tolerance and survival after two winters under field conditions. On average, the exotic germplasm displayed distinctly lower disease tolerance and final survival than the Italian germplasm, while showing a non-significant trend towards lower yield. Egyptian variety germplasm was superior to Egyptian landrace material in all respects. Although rare, outstanding Egyptian genotypes could be identified to synthesize a 10-parent Syn-2 population for use as a free-hybrid component. Two additional 10-parent Syn-2 populations representing candidate components were selected from the well-adapted Italian landrace and from a European semi-erect germplasm pool, respectively. Schemes for producing 2-way or 3-way free-hybrids from these populations are discussed.

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P85

F1² performance of tetraploid perennial ryegrass on the basis of the composition of a synthetic variety

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Abstract
Synthetics of tetraploid perennial ryegrass based on only 2 components may have a good herbage yield but mostly have a low seed yield (Baert et al., 2007). How can we use the performance results of multiplied F1 populations (F1²) to build synthetic varieties?
We carried out a diallel cross with 10 genotypes of tetraploid perennial ryegrass. The F1 seeds were multiplied to F1² seeds. We determined the dry matter yield of the F1² populations in a field trial for 2 years. The results showed that the genotypes with the best general combining ability produced the best F1² progenies. The best 3 F1² progenies were used to build a synthetic variety that is performing very well in official trials.
This example shows that the use of pair crosses in synthetic variety development of tetraploid varieties is valuable but not necessarily superior to other methods like polycoss or topcross where GCA is determined and mother clones are maintained.

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O15

Physiological and genetic diversity in *Rhizobium sullae* from Morocco

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Summary

Sulla is an important forage legume in the northern parts of Morocco and has a significant role in minimizing soil erosion. Sulla consists of two species *Hedysarum coronarium* L. and *H. flexuosum* L and both are well adapted to marginal areas where drought, salinity and alkalinity are the major problems. With an aim to characterize the bacterial symbiotic partner, in this study, *Rhizobium sullae* isolates from the nodules were sampled from sulla growing regions of Morocco. A total of 62 isolates were characterized so far, for various physiological characters such as resistance to salinity stress, water stress, high temperature stress, antibiotics, heavy metals, and various pH levels. The results revealed that there was a considerable diversity exists for various physiological traits and the 62 isolates were divided into 14 clusters which showed a wide range of diversity both within and between the clusters. Genetic diversity of the isolates was analyzed by Amplified Ribosomal DNA Restriction Analysis (ARDRA) using two enzymes *Hin*fI and *Hae*III. The dendrogram obtained shows the existence of eight independent lineage and 9 delimited clusters. Many strains belonging to the various physiological groups were also distributed in different genetic groups, indicating that they were genetically divergent strains.

Keywords: *Rhizobium sullae*; Physiological traits; ARDRA; Genetic diversity

P86

Seed yield of new *Festulolium* varieties bred from *F. glaucescens*

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Abstract

Since 2007, three tetraploid (2n = 4x = 28) cultivars using for the first time *F. glaucescens* as fescue progenitor were registered in the French National list of *Festulolium* (Ghesquière, 2007). We report a wide seed yield assessment of those varieties in comparison to 5 control *Festulolium* varieties, *i.e.* derived from *F. arundinacea* or *F. pratensis*, 10 control varieties of pure species and 12 experimental populations including the very initial F2 population of *L. multiflorum* x *F. glaucescens* hybrids from which all breeding polycrosses and new varieties have been produced. Among the 12 populations, 4 covered up to 3 generations of successive seed multiplications; also, 2 populations were bred following one generation of backross (BC1) either into 4x *L. multiflorum* or *L. perenne*. Genetics of seed yield was eventually approached by adding 212 Half-Sibs progenies (HS) covering all single entries of 15 polycrosses and distant, for the most advanced ones, by 10 generations (meiosis) since primary hybrids. Overall, the trial comprised 244 genotypes; it was sown by Oct. 2007 into a 2-replicates unbalanced random design of 3.1 m² single plots which was mechanically harvested on the 1st and the 3rd of July 2008 depending on maturity.

The highest seed yielding genotype was the control tetraploid Italian ryegrass (*Tonyl*) with 12.3 dt/ha. All pure fescue control varieties did not yield more than 5.1 dt/ha likely because of limiting vernalisation due to late sowing. The two Cvs *Lofa* and *Paulita* were the best yielding control *Festulolium* with 11.9 and 10.5 dt/ha (resp.), closely to previous seed yield evaluation (Fojtik, 1994). On average, populations and varieties from *F. glaucescens* yielded 6.8 dt/ha, not significantly different from the 3 other control *Festulolium* Cvs, *Felopa* (7.6), *Duo* (7.1) and *Lifema* (6.3). Total genetic variance among HS was estimated to be of 1.23 (dt/ha)², of which, 42 % was found between polycross and 58 % between HS within polycross, which suggests prospects of response to HS selection for increasing seed yield; however, significant genetic variance was found neither among populations of advanced breeding generations nor over seed multiplication generations. Similarly, no genetic improvement was evidenced since the initial F2 generation of primary hybrids (5.6 dt/ha). By contrast, the 2 BC1 populations had seed yield (11.0 dt/ha on average) significantly restored up to the level of the best *Festulolium* Cvs *Lofa* and *Paulita*.

Improvement in *F. glaucescens*-derived varieties as well as stability over generations is likely expected to come from allopolyploid of that fescue species and disomic inheritance promoted by high homologous chromosome pairing (Ghesquière et al, 1993). However, this may partly hamper normal development of albumen in complex polyploid seedlings as well as response to breeding despite large genetic variability and high heritability. This is in agreement with general default of seed yield in *F. arundinacea*-derived hybrids and, conversely, better potential from colchicine-induced autopolyploid *Festulolium* derived from *F. pratensis*. Nevertheless, higher fertility in these latter *Festulolium* could also come from continuous lost of fescue chromosomes over generations (Kopecky et al, 2005, Zwierzykowski et al, 2005). In this respect, it seems that genome balance compatible with potential for seed yield could be significantly achieved in *F. glaucescens*-derived *Festulolium* only following one generation of back-cross into *Lolium* sp.

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Production of self-fertile interspecific hybrids between *Lolium temulentum* x *Lolium multiflorum*

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Abstract

Lolium temulentum L., which is a self-fertile species, can hybridize with *L. perenne* L. and *L. multiflorum* Lam. relatively easily, but there is no report about the self -fertility of those interspecific hybrids. In this work, we investigated morphological traits and fertility of the diploid F_1 hybrids between *L. temulentum* x *L. multiflorum* (2n=14) and suceeded in getting self-fertile ones. Three F_1 hybrids between *L. temulentum* (Ba3081) and a genotype of *L. multiflorum* were obtained by embryo rescue technique according to Yamada (2002) and then proved to be interspecific hybrids analyzed by SSR markers of Italian ryegrass (Hirata *et a*l., 2006). We checked the viability of their pollen by 1% acetic carmine staining. Pollen viability of F_1 hybrids was considerably lower than that of parental plants, but normal pollens were observed in F_1 hybrids. To assess the potential for fertility and self-incompatibility in F_1 plants, each F_1 plant was self-pollinated in paper bag in the greenhouse. We obtained approximately 300 and 100 self-fertilized F_2 seeds from two F_1 hybrids, No.1and No.3, respectively. More than one hundred F_2 plants from the F_1 hybrid (No.1) have been raised in 2008 and their agronomical traits are now under investigation.

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P88

Introgression of novel traits into white clover (*Trifolium repens* L.) from related *Trifolium* species

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Abstract

Interspecific hybridization can be used to introduce traits into species where limited variation for that trait exists and in many species introgression from closely related species has been an important route to genetic improvement. Within the *Trifolium* genus, interspecific hybrids have been developed to introduce desirable traits into the agronomically important forage legume white clover (*T. repens* L.). Introgression of the rhizomatous trait from Caucasian clover (*Trifolium ambiguum* M.Bieb) into white clover (*T. repens*) has been carried out to improve persistence and tolerance of moisture stress (Marshall et al., 2001) and advanced backcross hybrids are now being submitted into official variety trials. Interspecific hybrids have also been developed between white clover and the annual, profuse flowering *T. nigrescens* (ball clover) as a strategy to improve the seed yield of white clover. Third generation backcross hybrids have now been developed in different leaf size categories and have the agronomic performance of white clover. Assessment of the seed yield of the hybrids (Marshall et al., 2008) showed that the medium and large leaved hybrids produced significantly more inflorescences and had a higher seed yield potential than control varieties of comparable leaf size improving the commercial potential of this material. Molecular and conventional breeding approaches, combined with extensive phenotypic analysis have been used to ensure that introgression of these novel traits has not detrimentally affected the agronomic.

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P89

Studies on the expression of exogenous CBF_4 gene in Transgenic wheatgrass

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Abstract:
Transgenic wheatgrass generated from hybrid wheatgrass (*Agropyron cristatum*×*A.desertorum cv.'Hycrest-Mengnong'*) were identified by PCR analysis and Southern blot. CBF_4,the exogenous gene,one of transcriptional factors plays an equivalent role in plant during drought adaptation and cold acclimation was transformed into wheatgrass with phosphinothricin acetyltransferase conferring herbicide resistance as selecting gene. Results of Northern blot assay displayed that exogenous gene CBF_4 expressed at transcription level in transgenic plants, further drought stress detection showed drought tolerance of transgenic plants were enhanced.
Key words: CBF_4 gene, Northern blot, drought resistance

O25

Heterotic response from a diallel analysis between lucerne cultivars of different geographic origin

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Abstract

A semihybrid variety development strategy could indentify natural hybrid vigor that exists between lucerne (*Medicago sativa* L.) germplasms, populations and cultivars. A successful semihybrid model is the identification of improved germplasm with superior agronomic traits as well as good combining ability between designated heterotic groups (Sakiroglu and Brummer, 2006). Identification of heterotic groups and patterns among breeding populations provides fundamental information to help lucerne breeders manipulate heterosis more knowledgeably (Segovia-Lerma et al., 2004).

A diallel analysis was conducted among five lucerne (*Medicago sativa* L.) cultivars, namely NS Banat ZMS II from Serbia, Zuzana from the Czech Republic, Ghareh Yon Geh from Iran, RSI 20 from Spain, and Pecy from France. The tested cultivars differed significantly in origin and phenotypic characteristics (Milić, 2007). A complete diallel cross including reciprocals was carried out. A spaced plant field was established in 2006 that included the five lucerne cultivars (parents) and their 20 diallel hybrids (F_1) sown in three replications, with 20 plants per replication.

Heterotic responses were determined by evaluating dry matter yield of the cultivars and their 20 diallel hybrids in seeded plots that were harvested five times in each of the two years of the study (2007 and 2008). The variation among crosses was attributed primarily to general combining ability (GCA) effects, while specific combining ability effects were not significant. A mid-parent heterosis [(MPH) range of −2.6 % to 25.4 %] and a high-parent heterosis [(HPH) range of −4.2 % to15 %] were detected. The hybrids between French and Spanish cultivars and those between Iranian and Spanish ones demonstrated the highest cross mean performance in diallel crosses. The results indicate that these crosses should be recognized as heterotic groups. The MPH results suggest that we may have capitalized heterotic response between divergent lucerne cultivars (different geographic origin) to improve lucerne forage yield.

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P90

Using bulk-hybrids for breeding adapted genotypes of subterranean clover

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Abstract
Changes were measured over 16 years in a self-regenerating, bulk-hybrid subterranean clover population, consisting of F_2 seed from 253 crosses, sown in the Mediterranean-like climate of south-western Australia at Nabawa (mean growing season length of 5 months) and Mt Barker (mean growing season length of 8 months). Seed bank samples harvested annually were grown in a common garden. Experiment 1 measured flowering time in plants from each year, while Experiment 2 measured 26 variables in the populations three and 16 years after sowing in comparison with the ancestral population. Changes in population means were observed in 20 characters and variability declined in 11 characters at one or both sites, with much of this occurring within three years (Nichols 2004). Natural selection at Nabawa favoured early flowering of long duration, thick peduncles, high harvest index and high hardseededness, while at Mt Barker it favoured late flowering of short duration, large leaves and long, thick petioles at flowering, thick stems with long internodes, long, thin peduncles with a high burial angle, large plants at maturity, low hardseededness and high biochanin A and total isoflavone contents. High seed production capacity, with high seed weight and seeds per burr, was important at both sites. The use of bulk-hybrid populations is suggested as a low-input means of breeding and selecting well-adapted subterranean clovers and other self-pollinated, self-regenerating annual pasture legumes.

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O24

Identification of heterotic patterns in perennial ryegrass

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Abstract

Perennial ryegrass (*Lolium perenne* L.) is spread all over Europe and ecotypes reflect the large amount of genetic diversity present. Breeders took profit from this diversity in creating new varieties. However, they intermated whatever materials they had available and largely ignored the maintenance of divergent materials. With few exceptions, nowdays varieties built just one single genepool. Furthermore, many ecotypes from Northwestern Europe belong to the same genepool like most varieties (Bolaric *et al*. 2005). Melchinger and Gumber (1998) outlined guidelines for the identification of heterotic groups and patterns. In brief, pre-grouping of the germplasm based on genetic similarity is followed by the evaluation of diallel crosses, and finally, selection of the most promising cross combinations as potential heterotic patterns. Important criteria are high *per se* performance of the parent populations and high mean performance and large genetic variation in the hybrid population.

Pre-grouping of 8 populations was done according to their geographic distance (160 to 1,800 km). The 8 parent populations and their 28 diallel crosses were evaluated for annual dry matter yield (ADMY) for two years at two contrasting locations. Mean performance of the parents was 13.6 t/ha ADMY compared to 14.1 t/ha ADMY of the hybrids . The most distant cross yielded highest (15.5 t/ha ADMY), which resulted in a mid-parent heterosis of around 13%. The association between geographic distance and hybrid performance with r=0.64 was much closer as the one between molecular distance and hybrid performance (r=0.27) found in a previous study (Posselt 2005).

These two populations fulfill the heterotic pattern criterion mentioned above and could be the nucleus of the heterotic groups to be established. The broadening of the respective groups will be outlined and discussed, as well as the application in practical plant breeding.

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Genetic Modification of Forage Grasses and Legumes

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Genetic engineering offers effective ways to improve forage grasses and legumes. We have established genetic transformation systems for a number of important forage and turf species including tall fescue (*Festuca arundinacea*), switchgrass (*Panicum virgatum*), bermudagrass (*Cynodon dactylon*), Russian wildrye (*Psathyrostachys juncea*), darnel ryegrass (*Lolium temulentum*), zoysiagrass (*Zoysia japonica*), alfalfa (*Medicago sativa*), white clover (*Trifolium repens*) and *Medicago truncatula*. The target traits are forage quality, drought tolerance, phosphate uptake and improved biofuel production. Forage quality, particularly digestibility, is a limiting factor for animal productivity. We cloned cDNAs of major enzymes involved in lignin biosynthesis and produced transgenic tall fescue plants that showed reduced lignin content, altered lignin composition and increased dry matter digestibility. Drought tolerance is an important trait for improvement in perennial forages. We characterized novel ERF transcription factor genes (*WXP1* and *WXP2*) from the model legume *M. truncatula.* Overexpression of the genes in alfalfa and white clover led to a significant increase in cuticular wax loading on leaves, decreased water loss and enhanced drought tolerance. Phosphorus is immobile and often deficient in pasture soils. Improving phosphate uptake in plants is an economic way to increase forage production. We cloned and characterized a constitutive promoter, two root-specific promoters, a novel phytase gene and a purple acid phosphatase gene from *M. truncatula*. Transgenic expression of the phytase gene or the purple acid phosphatase gene in Arabidopsis and white clover led to significant improvement in organic phosphorus uptake and plant growth. Switchgrass is an important bioenergy crop. Lignification of grass cell walls negatively affects enzymatic hydrolysis and utilization of structural polysaccharides. We have cloned major lignin genes from switchgrass and regenerated transgenic switchgrass plants with RNAi constructs of the lignin genes.

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Infection of perennial ryegrass by fungal diseases in northern climate

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Abstract
A field trial was performed in 2004-2006 in Jõgeva with the aim to select sources for disease resistance breeding from among perennial ryegrass (*Lolium perenne*) varieties bred in 9 European countries. Each of three maturity classes was represented by 5–6 diploid or tetraploid varieties. In addition to ordinary NPK-background, 400 kg ha^{-1} of magnesium fertiliser (Mg 15%, S 19%, Ca 3%) was applied before sowing to half of the plots as the podzolic soil was deficient in magnesium. Foliar infection was assessed 9 times during the testing cycle, the severity of snow mould just after the first winter. As a mean of three years, there were minor differences in the infection level with *Puccinia coronata* (2–4 pts, if 9 designates full infection). Intermediate and especially late types turned out to be more resistant than earlies at both fertlisation backgrounds. At added Mg Estonian cv 'Raidi' was scored with 5 pts. Mean infection with *Drechslera siccans* was 4 pts (range 3–5) at both treatments. Diploids were more infected than tetraploids. 'Arvicola' was significantly less (3 pts) infected at both NPK and additional Mg-backgrounds. On average of the testing years, Mg-fertiliser significantly (by 1,3 pts) enhanced the resistanse against crown rust but tended to reduce it against brown blight. The infection with *Fusarium nivale* was significantly severer in case of Mg supplement (4,3 pts) compared with NPK-fertilisation (3,0). The resistance against snow mould in local diploid variety was equal to two Swiss and a German variety. European perennial ryegrasses demonstrate superior resistance against the foliar diseases prevailing in a region with moderate pressure of fungal pathogens.

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Fodder's quality of green pea (*Pisum sativum* L.) and broad bean (*Vicia faba* L.) cropped in Haddej_Bouhedma (Centre of Tunisia)

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Abstract

In the south and the centre of Tunisia, agriculture is closely related to the availability of water. It is localized in oases, some irrigated perimeters, and in the traditional hydraulic constructions "Tabias and Jessers". Haddej-Bouhedma is located in the central of Tunisia known as the latest region of *Acacia raddiana*. In this area, agriculture is among the main economic activities. During rainy years and thanks to traditional water management, rainwater is collected and used for different plantations like legumes. The emphasis of this paper is to study the fodder's quality of two fixing nitrogen leguminous plants cropped in this region: the green pea (*Pisum sativum* L.), which is recently introduced to Haddej Bouhedma's region, and the broad bean (*Vicia faba* L.) and their role in the improvement of soils fertility of the region.
For that an in situ investigation and observations on open field have been achieved. Results indicate that both pea and broad bean allow the production of an interesting fresh biomass, fodder and nodules. It is noted also that the green pea develops a dry matter production in the aerial and in root parts as well as a number of nodules more elevated than bean. In addition, the two leguminous seems advantageous concerning enrichment of soil in nitrogen. In fact, measured in two plains, this content in the soils cropped with pea and bean is respectively, 16.9×10^{-5} % against 6.3×10^{-5} % in the first one and 8.1×10^{-5} % against 7×10^{-5} % in the second. This nitrogen could be very useful for the cereals of the coming season.

Key words: Haddej Bouhedma, green pea, broad bean, nitrogen.

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Effect of light quality and quantity on leaf growth in *Lolium perenne* L.

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Abstract
In a sward, the quantity and quality of light are modified by the canopy, with in particular a decrease of red – far red ratio and a decrease of blue light. Plants react to these modifications by altering their shoot morphogenesis. In particular, in grasses, modification of light composition induces leaf growth changes. The objective of this study was to evaluate the effect of light quality and quantity on leaf growth in a set of perennial ryegrass genotypes. Ten clones of ten perennial genotypes highly variable in leaf length were used. Four light treatments were applied: a standard treatment (transparent filter), decreased PAR (neutral filter decreasing all the wavelengths similarly), low red – far red ratio associated with low blue (green filter, simulating the effect of a canopy), and low blue (red filter). After a growing period of three weeks, plants were defoliated and the light treatments were applied. Leaves three and four following defoliation were measured in order to obtain leaf elongation rate (LER), leaf elongation duration (LED) and adult leaf length. Strong genotypic and light effects were observed on all traits. The low blue treatment had a particularly large effect, increasing adult leaf length by increasing both LER and LED. The differences in adult leaf length between genotypes were explained by both LER and LED. The genotypic x treatment interaction was significant but low in comparison to the principal effects.

P95

What are the cellular components underlying genetic diversity of leaf growth in *Lolium perenne* L.?

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Perennial grasses exhibit a large genetic variability in leaf growth and length (Sleper and Nelson, 1989; Ghesquière *et al.*, 1994). This variability in leaf growth has major consequences on productivity (Horst et al., 1978; Barre *et al.*, 2006), on morphology of forage/turf types (Barre *et al*., this volume), and on competition and dynamics of individual plants within populations (Hazard and Ghesquière, 1995). To orientate genetic analysis, the cellular components determining the variability in leaf growth were analysed in *Lolium perenne*. The study was conducted on 8 individuals of contrasted leaf length originating from a forage mapping population, and on a turf type. Plants were vegetatively propagated and grown in a cabinet under optimal conditions. Elongation of leaves 2 to 6 following defoliation was characterised. Epidermal cell number and cell size of mature leaves, as well as cellular dynamic components in the leaf growth zone at rapid elongation, were determined on leaf 6 (Schnyder *et al.*, 1990). Results showed that variability in mature leaf length is related to variability in cell number rather than cell size in the forage population, whereas both components are reduced in the turf type. The variability in mature leaf length was due to variability in rate rather than duration of leaf elongation. In the forage population, variability in leaf growth rate was due to variation in number of cells simultaneously elongating in the growth zone, due to variation in cell production rate, whereas elongation rate and duration of individual cells were similar. In the turf type, the lower leaf elongation rate was due to a decrease in both cell production and cell elongation rates. In conclusion, cell division plays a major role in genetic variation of leaf elongation among forage types, but cell elongation is also a determinant between turf and forage types.

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Effect of three rates of irrigation on five Lucerne (*Medicago sativa* L.) populations cropped in oases conditions

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Abstract
This research is reserved to study the effect of three rates of irrigation (R1:irrigation every 15 days, R2:irrigation every 30 days and R3:irrigation every 45 days) on 5 populations of alfalfa (*Medicago sativa* L.) respectively Chenchou, Chenini, Matouia, Elgalaa and Douz, in order to select the most powerful population in the oasis.
Results showed that:
- The rate **R1** (an irrigation every 15 days) affected positively the growth of stems length as well as the number of the sheets and increased the production of fresh and dry matter. The rate (**R3**) affected negatively these parameters.
- The application of a severe irrigation (irrigation every 45 days) increased length and width of the central leaflet.
- **The** rate **R3** increased the number of inflorescences and the number of flowers per plant, whereas it reduced the number of pods per plant and the number of seeds per pod.
- The increase of the period of irrigation reduced the phosphorus, sodium, potassium and nitrogen contents for all the populations.
Key words: alfalfa, irrigation, population, water stress

P97

Effect of different doses of compost based on tree palms waste on Lucerne (*Medicago sativa* L.) cropped in oases conditions

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Abstract

The Tunisian coastal oasis suffers from salinity, soil degradation and lack of fertility. In these oases the accumulation of date palm's wastes induces their pollution. Two experiments relating to: (i) Comparing two types of amendments (compost and manure) after two years (ii) and the effect of different doses of compost on the growth, yield and quality of alfalfa, were carried out. Results shows that: After two years (i) the number of plants per m² and yields have not been influenced by the two types of amendments but yields (resulting from ten cuts), expressed in dry matter are stable both for the two treatments .

The effect of different doses of compost (ii) noted that:

* Vegetative activity in winter is almost the same for all doses studied
* Growth of alfalfa in height is faster with the treatment 4 Kg of compost / m². This growth is much faster during the summer than during the winter period
* Leaf area and seeds production are influenced by the doses of compost applied, the dose 3 Kg / m² production's has generated the highest yield expressed on fresh and dry matter. The treatment 4 Kg / m² seem to correspond to a luxury consumption dose. For these two experiments, fodder production has got a good nutrition quality. It is rich in dry matter and well endowed with potassium, sodium, phosphorus and nitrogen.

Keywords: Oasis, compost, alfalfa, growth, yield and quality.

P98

Chemical Composition of first cut forage ryegrass (*Lolium*) species

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Ryegrass is one of the most widely grown grasses in the world. They have numerous desirable agronomic qualities. 4 ryegrass species: annual (*Lolium multiflorum* var. *westerwoldicum*), Italian (*Lolium multiflorum*), hybrid (*Lolium boucheanum*) and perennial (*Lolium perene*) were studied in the Lithuanian Institute of Agriculture during 2007 – 2008. Crude proteins (CP), water soluble carbohydrates (WSC), crude fibre (CF) and dry matter digestibility (DMD) were evaluated.
The Italian and hybrid ryegrass had significantly highest DMD (86.28 % and 85.56 %) and WSC (37.37 % and 35.98 %). These species also had low CF percent (18.8% and 19.33%). Annual ryegrass had significantly lowest DMD (62.21 %) and higher CF (26.15 %). Proportion of crude proteins and water soluble carbohydrates is a very important ryegrass characteristic. Annual ryegrass had the best proportion of CP and WSC – 0.83. This species also had significantly highest CP and lowest WSC among all studied species. Average CP variation was assessed in annual, Italian and hybrid ryegrass, and low CP variation – in perennial. Average WSC variation was estimated in annual ryegrass and low in all other studied species. The crude fibre and digestibility variation was low in all species. These ryegrass varieties were distinguished for the best chemical composition: annual– 'Elunaria', 'Weldra' and 'Energa'; Italian – 'Talvike', 'Delecta' and 'Corbes'; hybrid – 'Abereve', 'Agata' and 'Lorry'; perennial – 'Žvilgė' and 'Raminta'.

P99

Study of productivity and summer drought resistance of three perennial forage grass species (*Dactylis glomerata* L., *Festuca arundinacea* Schreb. et *Phalaris aquatica* Desf), in Metidja (Algeria)[6]

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Abstract
The scarcity of the rains in Mediterranean southern regions and their bad distribution in time and space are considered as the major problem of the fodder production and the pastoral activity in these environments (Abdelguerfi and Laouar, 2000). The three species wich are the subject of this study (tall fescue, cocksfoot and phalaris) can be a palliative for this limitation, throw their good productivity in favourable seasons and their ability to resist and survive the difficult environmental conditions (summer drought). In our trials and on two years (3rd and 4th year of growth), the best results have been obtained on tall fescue varieties with respectively 6.27 t DM.ha^{-1} and 5.56 t DM.ha^{-1}. For cocksfoot, the mean of the species is about 3.16 and 4 t DM.ha^{-1} for the two successive years. The most productive variety was Fletcha endophytée for tall Fescues (6.8 t DM.ha^{-1} in 4th year) and Kasbah for cocksfoot varieties (4.81 t DM.ha^{-1} in the 4th year). On both years, tall fescue shows the best water use efficiency (WUE) with 2.13 DM.m^{-3} and 3.95 kg DM.m^{-3}, while the cocksfoot values were lower (1.63 and 2 kg DM.m^{-3}, 57 kg DM.m^{-3} respectively on the two years). Perenniality on the 4th year was highest on tall fescue varieties with a species mean of 33.02 % and only 15.52 % on the cocksfoot ones. Fletcha endophytée is the most persistent tall fescue variety; it keeps 63.81% of its initial plants, and Ottava is the best one for cocksfoot (27.92%). These results confirm those of other works on forage grasses which demonstrate the best behaviour of tall fescue compared to cocksfoot (Norton et al., 2006; Volaire and Norton, 2006).

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O13

Comparison of different low-input lignocellulosic crops as feedstock for bio-ethanol production.

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Lignocellulosic biomass is a renewable carbon source and can significantly contribute to a reduction of the use of fossil resources for the production of energy, chemicals and materials. For example, lignocellulose serves as feedstock a.o. for the production of energy (by direct combustion or by fermentation), building materials (wood, thatch, straw, particle board), paper and cardboard.
In this study several low-input energy crops are compared for their potentiality as feedstock for bio-ethanol production. In May 2007, a yield trial was installed with the following crops: two *Miscanthus* species, two varieties of *Phalaris arundinaceae* (reed canary grass), two varieties of *Panicum virgatum* (switchgrass), one accession of *Phragmites australis* (common reed) and one willow (*Salix* spp.) cultivar as short rotation coppice reference. The yield trial is conducted under low input; no fertilizer is applied and the trial is harvested once a year in late winter, early spring. In 2008 and 2009, dry matter yield was determined as well as the cell wall composition (ADL, ADF and NDF). Two years' experience will be discussed.

O14

Development of *Melilotus siculus* – a new salt and waterlogging-tolerant annual fodder legume species for Mediterranean-type climates

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Abstract

In Australia 5.7 million hectares of agricultural land are currently affected by dryland salinity or at risk from shallow water-tables, with this area expected to rise to 17 million hectares by 2050 (National Land and Water Resources Audit 2001). Many of these soils are also subject to periods of waterlogging. The commonly sown pasture and fodder legumes in southern Australia, most notably *Trifolium subterraneum* L. (subterranean clover), are among the most sensitive plants to these conditions. A recent series of field experiments across southern Australia found that of 33 self-regenerating annual legumes, *Melilotus siculus* (Turra) Vitman ex B.D. Jacks was the only one productive and persistent beyond the first year on waterlogged, saline (0-10 cm EC_e levels in summer > 8 dS/m) sites (Nichols *et al.* 2008). The salinity and waterlogging tolerance of *M. siculus* in the vegetative phase has been confirmed by glasshouse experiments (Rogers *et al.* 2008), while Nichols *et al.* (2009) have shown mechanisms for salinity tolerance and avoidance in germinating seedlings. While *M. siculus* shows great promise, its commercialisation as a new fodder legume species for saline, waterlogged soils is impeded by lack of a *Rhizobium* strain able to nodulate regenerating plants under saline conditions (Charman *et al.* 2006). Current work is aimed at identifying a suitable *Rhizobium* strain. If successful, evaluation of *M. siculus* genotypes will commence, with the aim of developing a new cultivar.

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O16

Fiber content and plant development in *Festulolium*

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Abstract
Candidate cvs. (*Lolium perenne x Festuca pratensis*) at diploid or tetraploid level, marketed *Festulolium* cvs. of different parental origin (Hykor, Felopa) and Grindstad timothy (*Phleum pratense*) were established in 2006 at two coastal locations; Fjaler, West Norway (61°N), Bodø, North Norway (67°N). At each location trials with four cutting regimes from vegetative stage through flowering, were conducted in three replicates. In 2007 and 2008 the cultivars were cut individually according to developmental stage. From each plot fiber contents as NDF and indigestible NDF (INDF) were analysed both from total grass yield and from stem and leaf fractions. At 1st and 2nd cut, NDF and INDF in the new candidate cvs. was significantly ($P>0.05$) lower than in Hykor and Grindstad. The differences were highest in the leaf fraction in which Felopa and candidate cvs. contained only minor content of INDF. Hykor differed significantly from the other cvs. by high dw leaf:stem ratio during the growth season, and the candidate cvs. differed significantly from each other from 2nd cut with diverging leaf:stem ratio. At each individual cut the developmental stage was estimated according to a phenological scale (Moore et al. 1991) to understand the relation between forage quality and growth stage.
For testing wintering ability, yield potential and forage quality in different climatic conditions of Norway, field trials including 9 marketed cvs. and 7 candidate cvs. of *Festulolium* were established in 2006 at Fjaler, Bodø and inland location Hamar (60°N). Market cvs. (Paulita, Perun, Hykor) were highest yielding in the southern locations (Fjaler, Hamar) whereas candidate cvs., because of better wintering, performed best at the northern location (Bodø). Trials and practical use have found cvs. of *F. arundinacea* origin to be most persistent in Norwegian farming, however, new candidate cvs. are promising both for yield potentials and forage quality.

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P100

Grain Legumes Technology Transfer Platform (GL-TTP): a way to integrate basic science and plant breeding

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Abstract
An independent and non-profit international organisation, the Grain Legumes Technology Transfer Platform (GL-TTP) was created to facilitate the exploitation of the results of the EU FP6 Grain Legumes Integrated Project (GLIP, www.eugrainlegumes.org). The GL-TTP members belong to both public and private sector and constitute a network that evaluates the needs and constraints of grain and other legume breeding programmes, assesses the relevance of new technologies and discoveries for the grain legume industry, collects and disseminates appropriate information within and between research and industry, sets up public/private and research/industry partnerships, organises training workshops and proposes technology transfer projects. GL-TTP focuses on genomic technologies to characterise the genetic diversity, identifies new genes of agronomic interest, validates the function of candidate genes using high-throughput functional screens of mutagenised populations and generates molecular markers for breeders. The GL-TTP activities so far have been focused dominantly on temperate grain legumes, where it acts in parallel with the European Association for Grain Legume Research (AEP, http://www.grainlegumes.org) as two great driving forces with the same major task: mobilising the global grain legume community to the benefit of each of their members. At the same time, GL-TTP has already begun to make first contacts with the research communities dealing with sub-tropical and tropical grain legumes, soyabean, forage legumes, most notably clovers and lucerne, and legume trees, such as *Robinia*, bearing on mind the results of basic science that are equally important and useful for breeding all cultivated legume species. Among the priorities of the GL-TTP actions related to its promotion and the enlargement, integration and improved coordination of the whole legume research community is its contact and potential concerted actions with EUCARPIA Fodder Crops and Amenity Grasses Section and the European Grassland Federation in all topics related to forage legume research to the mutual benefit.

P101

Root Health Condition of Alfalfa in Kerquin Sandy Land in China

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Abstract: The results showed that yields ranged from 10.07-23.47t/hm^2, total root length ranged from 215.17-708.89mm, root surface area ranged from 93.61-717.06cm^2, volume ranged from 3.24-57.72cm^3. The root rot was correlated with growth years. Algonquin was suitable cultivar for Kerquin Sandy Land.

Key words: alfalfa; root system; health assessment

P102

The effect of cutting on yield and quality of winter wheat and triticale for grain and forage

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Abstract

Temperate cereals are grown predominantly as grain crops. However, the practice of grazing fall-sown winter cereals has been widelly known and conducted in many parts of the world with mediterranean type climate (Dunphy et al., 1982). In this study carried out over 2 years in experimental field of Faculty of Agriculture of Namik Kemal University of Turkey, the effects of cutting treatments (a. control, b. 1 time cutting. c. 2 times cutting and d. 3 times cutting) on seed yield and related characteristics, and quality of forage obtained by cutting of 2 winter wheat and 2 triticale cultivars were investigated. A split-plot design was used in the study. Grazing was simulated by lawn cutter. Based on the results of the study, cutting treatments reduced grain yield and related characteristics, and quality of the forage significantly. The average reduction in grain yield was 10% in only one time cut, 26% in 2 times cut and 82% in 3 times cut plots. The results of the study indicate that two months after sowing, winter wheat and triticale can be grazed one time in the years when there is a shortage of forage.

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Colletotrichum trifolii (Bain *et* Essary), anthracnose inducer on alfalfa in Serbia

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Abstract

Southern anthracnose or crown rot, caused by *Colletotrichum trifolii* (Bain *et* Essary), is a disease which has been detected on alfalfa in 9th decade of last century in Serbia. During three years period, especially in summer and autumn, plants with anthracnose symptoms were observed in alfalfa field. Stem infection results in wilting and death of the upper portion of the steam, giving rise to the characteristic "shepherd's crook" symptom. Strains of *C. trifolii* were isolated from diseased alfalfa stems collected from different locations in Serbia. According to preliminary pathogenicity examination four strains (Luc-7, Luc-17, Luc-27, Luc-33) were chosen for further investigations. Two methods were used to determine strains pathogenicity on commercial cultivar K-28. Infected plants showed symptoms of "shepherd's crook" in both causes. Phytopathological investigations of seed of four commercial alfalfa cultivars (K-28, NS Mediana, Affinity+Z and Alfagraze) were conducted also, by the agar-plate methods. Plants were classified for anthracnose reaction on 1 to 5 scales by Ostazeski *et* al. (1969). Investigated cultivars had different reactions to *C. trifolii* isolates. Alfalfa cultivar Alfagraze had the highest degree of healty plants, that ranged of 64,5% for strain Luc-27 to 56,3% for Luc-17. Resistance of other investigated varieties was strongly depending of *C. trifolii* strains. Isolates on PDA have developed olivaceous green to grey colonies with white to buff white margin, while substrate have got dark olivaceous green colour. Conidiophores were hyaline, vary in length, and produce a succession of conidia apically. Conidia were hyaline, straight, rounded at the ends, and nonsepted with average size 7,85x3,87 µm. Average sizes of appressoria were 7,5-16,5x5,5x8,9 µm. Molecular analyses, in comparison with referent strains CBS 158.83, have confirmed that the isolates Luc-7, Luc-17 and Luc-27 are *Colletotrichum trifolii.*

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Relations between site conditions and endophyte colonization of grasses in Poland

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Endophytes are fungi that lives symbiotically within the grass plant and produces toxins that confer disease and insect resistance to the host plant. These traits are beneficial for grasses which are grown in areas where insect and disease pressure may cause serious damages to their stands. However, endophyte toxins have also been linked with health disorders in livestock. For this reason, it is important to have knowledge on endophyte distribution as well as factors that favor grass colonization. The aim of our study was to discover relation between few site & climatic characteristic and pasture grass colonization by endophytes.

During 2007 and 2008 grass ecotypes were collected in a form of living plants from 186 localities around Poland. Total number of 464 ecotypes were gathered, mostly of red fescue (123 ecotypes) and meadow fescue (100 ecotypes).

For each ecotype ca. 5 plants were gathered and further planted in the field spaced plants nursery in Radzików. Plants were examined for endophyte presence using staining with aniline blue.

During ecotype collection following site characteristics were described: moisture intensity (1 – low, 2 – medium, 3 – high); usage intensity (1-9 scale where 1 is wasteland, 9 – intensively grazed pasture); soil type (1 – mineral, 2 – mineral/organic, 3 – organic). Also following climatic data were used: insolation (number of hours in year); yearly mean air temperature (degrees); yearly sum of rain (mm); length of vegetative season (days); length of summer duration (days) and length of winter duration (days).

The highest level of colonization was detected for meadow fescue (73%). More than a half (54%) of collected ecotypes of tall fescue were also colonized by endophytes. Relatively low levels of colonization were observed for tufted hairgrass (17.5%), sheep's fescue (16.7%) and smooth-stalked meadow-grass (7.9%). Statistical analysis yielded no significant correlation between endophyte colonization intensity and site characteristics. For climatic data significant and negative correlation was found for yearly mean air temperature (-0.69 **) and significant positive correlation for length of winter (0.80 **).

General conclusion is that average level of colonization (mostly meadow and tall fescue ecotypes) was the highest in regions of the lowest yearly temperature and long winter. It could be further suggested that the presence of endophytes is advantageous for plants in the face of winter conditions.

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